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GAS IN EVAPORATED MILK DUE TO A STREPTOCOCCUS

By B. W. HAMMER

From the Laboratory of Dairy Bacteriology, Iowa State College.

Received for publication August 15, 1927.

The organisms causing bacterial changes in canned evaporated milk are often easily isolated because of their presence in the spoiled material in pure culture. The usual absence of other organisms is due to the fact that the heat to which the milk is subjected destroys all except the more resistant types so that unimportant but confusing species are not present as they commonly are in raw milk or cream which has undergone some abnormal change. Spoilage in evaporated milk may involve all or a large portion of a given batch or only an occasional can. The present report deals with a change of the latter type in which the evaporated milk showed gas formation as a result of the growth in impure culture of an organism that was easily destroyed by heat.

HISTORY OF ABNORMAL MILK

The can of milk showing the abnormal condition was received from a condensery that had encountered comparatively little spoiled milk, and was of special interest because it came from a batch with which there was only a very small amount of spoilage. There was no suggestion of an extensive gas formation and the can showed only a medium amount of bulging at the ends, although it was received through the mail and was held in the laboratory for several days so that there was a good opportunity for bacterial growth. The milk showed no coagulation; the odor and flavor were somewhat abnormal but not particularly objectionable.

EXPERIMENTAL

Isolation of causative organism. The can was opened by treating a small area with concentrated HCl, driving this off after a few minutes with a flame and then puncturing in the treated area with a large nail that had been thoroughly flamed. When the tin was broken the escape of gas was very evident.

Plate and slope cultures were prepared with both standard and beef infusion agars, using room temperature and 37° C. for incubation. The plates at either temperature soon showed two types of colonies which were quite easily distinguished because one was more raised and more opaque than the other. The organisms forming the opaque colonies were gram positive cocci, while those forming the other type were gram negative rods. The slope cultures also readily showed these two types when stained mounts were prepared. There seemed to be no difference between the standard and beef infusion agars in the suitability for growth of the organisms.

The organisms were tried out for their ability to produce gas in evaporated milk by opening a can under aseptic conditions, inoculating and then

sealing with solder. Trials showed that the cocci regularly produced gas, while the rods did not. The gas produced by the cocci resulted in a definite bulging of the cans at the ends, but never in the bursting of a can.

Microscopic preparations of the cultures of cocci suggested that they were not pure because there were both chains and very definite clumps of organisms; an unusual variation in the size of the cells was likewise evident. Cans of evaporated milk which had been inoculated with some of the original coccus cultures were plated out, and, after incubation for several days at room temperature, the plates showed two types of colonies, a large opaque colony comparable to those secured with the original milk and a small, slowly developing colony; the former was a micrococcus and the latter, which was much more numerous, was a streptococcus. A series of inoculation experiments was then carried out using the micrococcus alone, the streptococcus alone, and the two in combination and showed that the streptococcus was responsible for the gas formation in the evaporated milk, since this organism either alone or in combination with the micrococcus regularly caused a bulging of the inoculated cans, while the micrococcus alone did not. It seems probable that colonies of the streptococcus were missed in the plates poured with the original milk because of their slow development and that the streptococci commonly contaminated the micrococcus colonies, due to the much greater numbers of the former.

Production of gas in evaporated milk. Gas was regularly produced in evaporated milk at both 37° C. and room temperature by inoculating the streptococcus either alone or in combination with the micrococcus. At 37° C. a bulging of an inoculated can was usually evident in from 36 to 48 hours, while at room temperature about 72 hours were commonly required. The presence of the micrococcus seemed to have no definite influence on the gas production of the streptococcus.

When a can showing definite bulging, as a result of a recent inoculation with the streptococcus, was shaken the swelling seemed to become more pronounced, presumably due to a liberation of the gas. In none of the inoculation experiments was the pressure developed sufficient to cause the bursting of a can. On opening a bulged can there was a definite escape of gas and often a small quantity of milk was forced out. In some instances, especially with recent inoculations, the fermented milk foamed slowly through the opening in the tin for considerable periods in quite the way typical foamy cream leaves a container.

Other changes produced in evaporated milk. Cans of milk which had been fermented by the streptococcus showed a definitely acid odor and a taste that was both sour and slightly bitter. The odor and taste were never very objectionable even after long holding periods at temperatures favorable for growth. The acidities developed in evaporated milk were commonly between 0.7 and 0.8%, usually about 0.75%, calculated as lactic acid, while the acidities of the uninoculated checks were about 0.45%. A definite coagulation of the inoculated evaporated milk was never observed, although a small amount of curd was sometimes noted along the walls of the cans.

Organisms in inoculated evaporated milk. Evaporated milk in which the streptococcus had grown contained surprisingly large numbers of organ-

isms. Stained mounts made within a short time following inoculation and incubation at a favorable temperature showed numbers of cells quite like the numbers present in starters; the organisms were arranged in pairs and short chains, the longest chains observed having from 15 to 20 cells per chain. In milk that had been inoculated longer, the number of gram positive cells seemed to be less and the individual cells appeared smaller, probably due to a dying out and partial autolysis of the organisms. Plate counts made on recently inoculated milk using the standard agar for milk analysis and an incubation of from 5 to 6 days at room temperature showed large numbers of organisms; some of the typical results are as follows:

CONDITIONS OF HOLDING EVAPORATED MILK

Temperature	Time	Bacteria per c.c.
37° C.	2 days.....	810,000,000
37° C.	2 days.....	670,000,000
37° C.	8 days.....	1,540,000,000

The plate counts, like the microscopic preparations, suggested that the organisms died out fairly soon in cans of evaporated milk, since with the milk inoculated for some time the numbers of colonies developing on standard agar plates were much smaller than the counts given above.

When the streptococcus was inoculated in combination with the micrococcus, the fermented milk on examination by the plate method showed much larger numbers of the former organism than of the latter. The same relationship was suggested by the microscopic preparations made, although there was always the possibility that with this method the two types were not accurately distinguished.

Gas production in ordinary milk in tubes. The streptococcus responsible for the gas production in evaporated milk did not show the definite gassy condition in tubes of plain or litmus milk that a typical gas forming organism does and the usual observations on tubes of milk gave no indication of gas. However, the development of gas in ordinary milk was shown by sealing freshly inoculated tubes of milk with a mixture of paraffin and vaseline (about 1 to 2 by weight); in such trials a small amount of gas ordinarily developed just beneath the seal, while the uninoculated check and cultures of the micrococcus showed no such development. The gas produced was, however, surprisingly small in amount and this probably explains why the gas cannot be detected without sealing the milk in which the organism grows.

Characters of the causative organism. In studying the causative organism from the standpoint of characters that might be important in its description and identification, considerable volatile acid production in milk was noted. Tests were accordingly made to determine the effect of added citric acid on the volatile acid production so that any relationship to the citric acid fermenting streptococci might be detected. The volatile acidity was determined in milk alone and, for comparison, in milk from the same lot to which sterile citric acid solution (equivalent to 0.2% crystallized citric acid) had been added at the time of inoculation; the incubation was seven days at room temperature. The following representative data show the general trend of the results:

	Volatile acidity* in milk alone	Volatile acidity* in milk plus citric acid
Culture 1	20.6	22.5
Culture 2	21.4	21.7

When a larger percent of citric acid was added to the milk the volatile acid production was greatly decreased, due presumably to a suppression of the organism by the acid. Trials made at the same time with cultures of *S. paracitrovorus* showed the usual pronounced increase in the volatile acidity when citric acid was added to the milk. A number of comparisons of the volatile acid production of the isolated organism with and without added lactic acid were also made, using 1 c.c. of sterile lactic acid U. S. P. VIII for 325 c.c. of milk; representative results are as follows:

	Volatile acidity in milk alone	Volatile acidity in milk plus citric acid
Culture 1	23.8	15.2
Culture 2	17.9	14.7

From the results presented it seems evident that neither citric nor lactic acids are the source of the volatile acid formed and accordingly that the streptococcus isolated does not produce volatile acid from the materials used by *S. citrovorus* and *S. paracitrovorus*.

Production of gas in evaporated milk by S. citrovorus and S. paracitrovorus. Because the causative organism produced considerable volatile acid resembling *S. citrovorus* and *S. paracitrovorus* in this respect, trials were carried out to determine whether or not the latter types would produce a swelling of cans of evaporated milk into which they were inoculated. *S. citrovorus* was tried at room temperature only and *S. paracitrovorus* at room temperature and also at 37° C.; with both organisms the attempts were successful with all of the considerable numbers of cultures tried. The time required for a bulging of the cans was essentially the same as the time required by the organism isolated from the spoiled evaporated milk. When a small hole was punched in one of the cans after *S. citrovorus* had grown in it, considerable gas escaped; no coagulation of the milk was noted. When the cans inoculated with *S. paracitrovorus* were punctured, there was a very vigorous escape of gas and the milk, which was usually curdled, was sometimes thrown for several feet. Only one of the cans inoculated with *S. citrovorus* or *S. paracitrovorus* was blown open and this occurred with *S. paracitrovorus* at 37° C.; the time required was 54 days.

*The method of determining the volatile acidity was that repeatedly used by this station. See Ia. Agr. Expt. Sta. Res. Bul. 63. The figures given are the c.c. of n/10 NaOH required to neutralize the first liter of distillate secured when 250 gms. of the fermented milk were distilled with steam after the addition of 15 c.c. of n/1 H₂SO₄.

DESCRIPTION OF THE ORGANISM, *STREPTOCOCCUS DISTENDENS* SP. NOV.

The organism is believed to be an undescribed species and the name *Streptococcus distendens* is proposed for it. A description follows:

MORPHOLOGY:

Form: The organism was spherical; the elongation of the cells commonly noted with streptococci was not as evident as it is with the usual *S. lactis* cultures.

Size: The diameters of the cells varied from about 0.7 to about 0.9 microns.

Arrangement: The organisms appeared singly, in pairs, and in short chains; the longest chains observed were made up of from 15 to 20 cells each. There seemed to be slightly more chain formation in milk than on agar.

Motility: There was no evidence of motility in hanging drop preparations, even when young vigorous bouillon cultures were studied.

Staining reaction: The organism stained readily with the usual stains. It was gram positive, although negative cells were common.

Spore formation: Nothing resembling spores was seen in microscopic preparations. In trials in which the organism was heated in milk, it was destroyed by 60° C. for 15 minutes.

CULTURAL CHARACTERISTICS:

Beef extract agar slope: Growth was usually evident after 24 hours at room temperature as tiny distinct colonies. These later grew together, but the growth always remained small in amount. It was white, smooth edged, only very slightly raised and non-viscous.

Whey agar slope: Growth was essentially the same as on a beef extract agar slope.

Beef extract agar stab: Growth was usually evident after 24 hours at room temperature and increased somewhat with age. The fully developed colonies were small, flat, white, smooth edged and non-viscous.

Agar colony: The colonies were usually evident after 24 hours at room temperature and increased somewhat with age. The fully developed colonies were small, flat, white, smooth edged and non-viscous.

Whey gelatin stab: A white uniform growth occurred along the entire line of inoculation but there was no surface growth. Liquefaction did not occur even after one month at 21° C.

Bouillons: Bouillon cultures showed a turbidity and sediment with no pellicle or ring at the surface. Growth was heavier in bouillons containing additions which the organism could ferment than in plain bouillon.

Potato: No growth was observed.

Dunham's sol: The organism produced a slight turbidity and sediment with no evidence of a pellicle or ring at the surface.

Uchinsky's sol: No growth was observed.

Litmus milk: Litmus milk was slowly reddened and later there was some reduction in the bottoms of the tubes. Coagulation was never observed.

BIO-CHEMICAL FEATURES:

Gas production: A small amount of gas was produced in evaporated milk and also in normal milk.

Oxygen relation: The organism was facultative.

Indol production: Indol was not detected.

Reaction change: Acid was produced in evaporated milk and a small amount in normal milk. Acid was also produced in bouillon containing fructose, galactose, glucose, maltose or sucrose and to a less extent in bouillon containing mannitol, lactose, salicin or inulin. There was no acid with glycerol, dulcitol, or raffinose.

Volatile acid production: Considerable volatile acid was produced in milk but the amount was not increased by adding either citric or lactic acids.

DISCUSSION OF RESULTS

The development of gas in evaporated milk by typical streptococci seems rather surprising because of the general characters of these organisms. However, Savage and Hunwicke¹ have reported both diplococci and streptococci as the cause of gas formation in the evaporated milk studied by them. The typical outbreaks of gas production in evaporated milk are most commonly due to anaerobic spore-formers that are, of course, quite resistant to heat and only occasional cans of gassy evaporated milk are encountered in which the causative organism is not resistant; it seems that these forms would have been destroyed if they had been subjected to any attempt at sterilization and accordingly it is often assumed that they get into the milk after the heating through some defect in the tin.

The organism isolated apparently does not produce the large amount of gas in evaporated milk that certain organisms do since the cans were never broken. In milk that had not been concentrated a comparatively small amount of gas was likewise produced and this was detected only by the use of a seal. It is probable that if the organism had not been isolated from gassy material, its gas production would not have been noted. This suggests that inoculation into cans of evaporated milk followed by proper sealing may be a useful method of determining gas formation in this material by organisms producing only small amounts of gas. Such a procedure showed gas formation with *S. citrovorus* and *S. paracitrovorus*, two organisms whose gas formation is not usually very evident.

The low resistance of the organism to heat indicates that it will never be of importance in evaporated milk and will probably be the cause of spoilage in only an occasional can when contamination occurs through some defect in the tin. It is of interest in this connection to note that milk from which the gas former was isolated also contained other non-spore forming organisms.

Considerable volatile acid was produced in milk by the causative organism but this apparently did not come from citric acid or lactic acid and the organism accordingly is not closely related to *S. citrovorus* or *S. paracitrovorus* as the volatile acidity developed in milk might suggest. The production of volatile acid from sources other than the sources used by *S. paracitrovorus* shows the importance of knowing rather definitely what materials are being fermented to produce volatile acid and also the danger of assuming that two streptococci are the same simply because they have the same general characters and both produce considerable volatile acid.

It has not been possible to describe the organism so that it can be identified with any degree of certainty, although the production of volatile acid from some source other than citric or lactic acids is of interest in this connection. There is a probability that it is the same as one of the organisms studied by Savage and Hunwicke, but this can not be definitely established. The organism is believed to be a new species and the name *Streptococcus distendens* is accordingly proposed for it, although it is appreciated that descriptions based on cultures from only one source are not entirely satisfactory and can not show the variations that are to be expected within the species.

¹Savage, W. F., and Hunwicke, R. F., Studies in unsweetened condensed milk. Food Insp. Bd. London. Spec. Rept. 13. 1923.

SUMMARY

A can of evaporated milk which had developed some bulging was studied and the abnormal condition found to be due to a streptococcus. The milk came from a batch showing very little spoilage and since other non-spore forming organisms were present in the milk it seems probable that there was contamination after sterilization through some defect in the tin.

The outstanding character noted with the streptococcus is the production of considerable volatile acid which apparently does not come from citric or lactic acids. The organism is believed to be a new species and the designation *Streptococcus distendens* has been suggested for it.

INHERITANCE OF CHEMICAL CHARACTERS IN MAIZE

E. W. LINDSTROM and FISK GERHARDT¹

Received for publication July 6, 1927.

In a former report (Lindstrom and Gerhardt 1926) there was given a discussion of the inheritance of total sugars, dextrin, starch and fat in the seed arising from crosses of sweet and dent corn. After this work was done, it seemed advisable to consider other chemical characters that might differentiate these types of maize, particularly since excellent genetic material was readily available. Accordingly chemical analyses of pentose, nitrogen and ash were included along with those of total sugars, dextrin, starch and fat.

Methods similar to those noted in the previous report were used with respect to handling the material from the genetic standpoint. The material consisted of the P_1 , F_1 , F_2 and backcross generations of a cross of Illinois High Fat² (white dent corn) by Evergreen sweet corn. The hybridization work was all controlled by careful hand-pollination methods. The parental varieties were inbred for only one or two years, since this is the only certain method of maintaining the varietal characteristics. Intensive or long continued inbreeding is almost certain to isolate strains differing widely from the general average of the parental variety. Even one or two generations of inbreeding have such a tendency, and care must be taken in selecting representative strains.

For the chemical analyses two lots of kernels were used, one comprising the entire kernel (pericarp, endosperm and embryo), the other with the embryo removed, leaving only the pericarp and endosperm. In the latter group the embryo or germ was carefully dug out of the dry kernel with a scalpel, since any soaking process would certainly change the chemical constituents of the grain.

METHOD OF CHEMICAL ANALYSIS

The air-dry corn samples were ground in an "Enterprise" and "Merker" mill respectively until the ground product could be passed through a 100 mesh sieve.

Moisture Determination: A tared sample of the ground tissue was dried to constant weight in a Freis electric vacuum oven at 70° C., thus permitting a rapid desiccation without the usual losses due to caramelization at higher temperatures.

Fat: The residue from the moisture determination was freed from lipoids and soluble pigments by percolation with anhydrous alcohol-free ether. The ethereal extract was dried to constant weight in an electric oven at 100° C., and expressed as percentage fat.

Total Sugars: After expulsion of the ether the residue of the sample was extracted with boiling 90 percent alcohol for one-half hour. The fil-

¹A cooperative project between the Genetics and Chemistry Sections of the Iowa Agricultural Experiment Station.

²The original material for the strains of the High Fat corn was obtained from Dr. C. M. Woodworth of the University of Illinois. He kindly provided three open pollinated ears of this selection showing analyses of 10.15, 10.31 and 10.47 percent fat.

tered extract was concentrated for removal of alcohol and made up to definite volume. An aliquot was acidulated with HCl to a final concentration of 2.5 percent and hydrolyzed by refluxing on an electric hot plate for two hours. After neutralization, the hydrolysate was made up to volume. Aliquots were clarified by use of neutral lead acetate and freed from excess lead by the use of sodium carbonate. The sugars present in the clarified aliquots were determined by use of the Defren-O'Sullivan (1896) method and expressed as glucose.

Dextrin: The residue from the sugar extraction was treated with 10 percent alcohol for thirty minutes at 50° C. for removal of dextrans. The concentrated filtrate was made to volume and hydrolyzed by use of 2.5 percent HCl in the usual manner. The neutralized hydrolysate was made to volume. Aliquots were clarified as in determination of total sugars. The glucose value was obtained by use of the Defren-O'Sullivan method, multiplied by the dextrin equivalent and expressed as percentage dextrin.

Starch: The residue from the dextrin extraction was boiled with 150 c.c. of H₂O for one minute. This was done to gelatinize the starch. After cooling to 38° C. and digesting with fresh saliva until a negative result was obtained with iodine, the filtered solution was hydrolyzed with 2½ percent HCl for one and a half hours. The glucose was determined by the Defren-O'Sullivan method, multiplied by the starch equivalent and expressed as percentage of starch.

Ash: A two gram sample was ashed, at a low temperature in an electric furnace, to a constant weight.

Total Nitrogen Expressed as Protein: The total nitrogen present in a two gram sample was determined by the Kjeldahl method multiplied by the factor 6.25 and represents total nitrogen expressed as protein.

Pentose: Two gram samples were distilled with 12 percent HCl according to the Official Methods (1920). The furfural phloroglucid value was multiplied by the proper equivalent and expressed as percent pentose.

In the subsequent tables all percentages are calculated on a moisture-free basis.

EXPERIMENTAL DATA

In Table 1 there are arranged the analyses of the eight chemical constituents of the parental types, showing to what extent the Evergreen sweet corn differs from the Illinois High Fat dent corn. The differences are particularly striking in the carbohydrate characters and to a lesser extent in the nitrogen, fat and ash characters. It is interesting to note that the carbohydrate analyses parallel each other in the material from the whole kernel as compared with that from the embryo-less grains. This merely indicates that the greater share of these carbohydrates resides in the endosperm.

This is not true, however, of the nitrogen and fat determinations. Here there is a reversal in the two lots since in the whole-kernel lot the dent corn contains the higher percentages of nitrogen and fat, whereas in the germless material the sweet corn has the greater amounts. The obvious explanation is that the embryo is the place where these constituents (fat and nitrogen) exist in the greatest amount. It is also well known that the Illinois

TABLE I. SHOWING DIFFERENCES IN CHEMICAL CHARACTERS BETWEEN THE PARENTAL STRAINS.

Percentages on moisture free basis.

	Whole kernel Average of three selfed ears		Embryo removed One sample from selfed ear	
	Dent (HF)	Sweet (Evergreen)	Dent (HF)	Sweet (Evergreen)
Total sugars	2.3	5.0	0.8	5.0
Dextrin	1.9	24.5	2.5	31.1
Starch	56.6	27.9	69.2	36.6
Pentose	6.7	10.2	4.9	9.0
Protein	14.3	11.2	9.2	12.1
Fat	9.4	8.0	0.9	1.7
Ash	1.6	1.8	1.6	1.7
Moisture ¹	6.6	5.5	8.9	6.8

¹Determinations on the air-dried material.

High Fat selections or strains are high in this constituent, primarily because of the relatively larger size of the embryo, which has been increased by a long period of selection for high fat percentage. Ordinarily sweet corns possess a higher fat value than dent corns, which is perhaps directly traceable to the fact that the sweet corn endosperm, being much lighter in weight than the dent type of endosperm, permits the embryo with its high fat content to bulk larger in proportion. When the germ is removed from the sweet corn, however, there is still a higher percentage of fat than in the germ-less dent kernels. Either the endosperm of sweet corn naturally carries a higher fat content, or what is equally likely, this type of endosperm absorbs more of the fat from the adjacent embryo.

TOTAL SUGARS, DEXTRIN AND STARCH

The hybrid generations arising from the Evergreen-High Fat crosses have been arranged in Table 2, where the main carbohydrate characters (total sugars, dextrin and starch) that differentiate sweet and dent corn are being considered. The carbohydrate index (CI) in this table is used to express the ratio of the water-soluble constituents (total sugars plus dextrin) to the water-insoluble form, starch. Apparently these three forms of carbohydrates in maize are interrelated since there is a decided tendency for the kernels to carry a similar, total carbohydrate makeup whether it be high or low in sugar, dextrin or starch. In other words, a low sugar content is in general balanced by a high starch content and vice versa.

There is a good parallelism between the analyses from the whole kernels and from the embryo-less grains, indicating that the endosperm tissue carries the major share of these carbohydrates. Accordingly there is no need of discussing the two types of analyses in Table 2 separately.

From the phenotypic viewpoint, it is of course well established that dent and sweet corn differ in a single pair of genetic factors (*Su su*)¹. The

¹For simplicity, the biliteral symbol *Su* has been replaced by the single letter *S*, representing the starchy gene or factor the allelomorph of *s*, the common sugary gene.

TABLE 2. CARBOHYDRATE PERCENTAGES IN PARENTAL, F₁, F₂ AND BACKCROSS GENOTYPES OF THE KERNEL.
Moisture-free basis.

Endosperm genotype	Whole kernel					Embryo removed				
	No. Samples	Total Sugar	Dextrin	Starch	CI	No. Samples	Total Sugar	Dextrin	Starch	CI
High Fat	3	2.3	1.9	56.6	.07	1	0.8	2.5	69.2	.05
Evergreen	3	4.9	24.5	27.9	1.01	1	5.0	31.1	36.6	.99
F ₁ (HF x E)	4	2.7	2.1	53.6	.09	2	2.4	2.3	63.7	.07
F ₁ (HF x HF)	1	3.6	1.7	56.1	.09					
F ₁ E x HF)	4	1.8	1.6	57.5	.06	1	0.6	2.0	74.6	.03
F ₁ x E	3	5.3	25.7	23.1	1.34	1	3.2	38.5	30.0	1.39
E x F ₁	3	2.2	2.2	55.8	.08	1	0.7	3.9	70.4	.06
	2	4.5	25.9	27.1	1.12	1	3.3	31.9	35.6	.99
S	4	2.3	2.7	57.5	.09	1	0.8	3.3	65.4	.06
F ₂	3	5.3	29.1	21.6	1.59	1	3.9	27.0	38.6	.80

F₁ generation of a dent-sweet corn cross shows the dominant starchy kernel. The F₂ hybrid seed generation (borne on the F₁ plants) exhibits the cleaneut segregation of the two kernel types on the same ear (75 percent starchy and 25 percent sweet or wrinkled). The backcross generation (F₁ x sweet corn), made reciprocally, consists of a 50:50 ratio of these kernel types on the same ear. The fact that two phenotypes appear on the same ear provides ideal conditions for eliminating, to a high degree, any environmental influences, thus making genetic differences easily demonstrable.

It may also be recalled that, due to the phenomenon of triple fusion of the nuclei in the fertilization of the maize seed, the endosperm may consist of any one of the following genotypes:

SSS—three starchy factors from both parents

SSs—two starchy from female and one sugary from male parent

ssS—two sugary from female and one starchy from male parent

sss—Three sugary factors from both parents

The above genotypes also may well represent an ordinary F₂ generation of a sweet-dent cross, giving the phenotypic ratio of 3 starchy to 1 sugary. The backcross genotypes of the reciprocal backcrosses of the F₁ hybrids to the recessive sweet corn would be as follows:

<i>F₁Ss</i> x <i>ss</i> ♂		
Starchy kernels	<i>SSs</i>
Sugary kernels	<i>sss</i>
<i>ss</i> x <i>F₁Ss</i> ♂		
Starchy kernels	<i>ssS</i>
Sugary kernels	<i>sss</i>

All of these various genotypes and phenotypes appear in Table 2.

There is some evidence of the cumulative effect of these endosperm factors when taken as a whole. True, there are some minor exceptions, but from previous experience with the chemical analyses, particularly those based on a few samples, these unexplained deviations are undoubtedly traceable to variations in the technique of present carbohydrate analysis as well as to fluctuations in the material.

Taking the carbohydrate indices from Table 2, the cumulative effect may be illustrated as follows:

Endosperm genotype	Whole kernel	Embryo removed
<i>SSS</i>07.....	.05
<i>SSs</i>07.....	.05
<i>ssS</i>09.....	.06
<i>sss</i>	1.24.....	1.04

Apparently there is no difference between the *SSS* and *SSs* genotypes in this material, but the influence of the two sugary factors in the *ssS* genotypes is felt in the increase of the sugars and dextrans, making for a small increase in the carbohydrate index.

It is worth noting in Table 2 that the sugar or dextrin content of the sweet corn which has been reisolated from dent (F₁) ancestry is just as

high as that of the pure parental sweet corn. This is clearly shown in the carbohydrate indices, the parental (Evergreen) index being 1.01 (in the whole-kernel lot) whereas the F_2 sweet kernels show the higher index of 1.59 and the backcross sweet kernels have indices of 1.34 and 1.12. From this it follows that sweet corn may be crossed with field corn, and from the cross can be reisolated sweet corn with a sugar and dextrin content high enough for all practical purposes.

The surprising thing is that these carbohydrates maintain such a constant interrelationship even when upset by radical hybridization methods. That they are so well controlled by the simple, Mendelian mechanism of genetic factors is evident in the consistent fact that the difference between the starchy and sugary segregates in both the F_2 and backcross generations is in all cases greater than the original parental difference. This is easily observed in a consideration of the carbohydrate indices in the whole-kernel group, as follows:

Parental difference in carbohydrate index.....	.94
F_2 difference between starchy and sugary types.....	1.50
Backcross difference— F_1 x Evergreen.....	1.28
Backcross difference—Evergreen x F_1	1.04

The differences are less marked in the analyses from the embryo-less kernels, but here the number of samples in any one comparison is too small to be significant.

What is true of this carbohydrate index, is equally true of the constituent parts of this index (total sugars, dextrin and starch). These greater differences argue strongly for the fact that the carbohydrate development in the corn grain is controlled by the single pair of Mendelian genes, since any greater genetical complexity would certainly cause a smaller difference to accrue in the later hybrid generations from a cross.

These findings with respect to the inheritance of the carbohydrate characters (total sugars, dextrin and starch) in this material afford a good verification for the work reported previously (Lindstrom and Gerhardt, 1926) on the same chemical characters, but involving different varieties of maize.

FAT

In Table 3, the chemical analyses for fat and nitrogen are arranged in a manner similar to that in the previous table. The same endosperm genotypes (determined by the genetic factors controlling the carbohydrate situation) are separated for analysis, and form a convenient method of presenting the data.

The interesting feature of this table lies in the fact that the parental dent corn (Illinois High Fat) of this cross contributed the higher percentage of fat. Usually dent types are lower than sweet types in fat. This difference in favor of the starchy (dent) type does not persist, however, since in later progenies, both F_2 and backcross generations, the recovered sweet kernels possess a significantly higher fat value. In fact, the F_2 sweet segregates (whole kernel) show an exceedingly high fat percentage, 11.3, higher than the original parent, and higher than commercial sweet corn varieties. This means that the high fat value of the dent type has been

incorporated into the sweet corn, and magnified because of the structure and relative endosperm-weight of the sweet corn.

A summary of the fat percentages according to the endosperm genotypes is given by the following:

	Whole kernel	Embryo removed
<i>SSS</i>	9.4.....	0.9
<i>SSs</i>	8.0.....	0.7
<i>ssS</i>	6.4.....	0.4
<i>sss</i> ¹	10.4.....	2.3

¹Does not include the parental *sss* type.

From previous work, one would expect the *SSs* and *ssS* type of kernels to be intermediate in value and approximately equal since there should be no appreciable cumulative effect of factors for the fat values that are based primarily on the embryo and its relative size. Any direct influence of the carbohydrate factors (particularly since they might change the relative weight of the endosperm) should give an ascending series of fat values among the starchy phenotypes (*SSS*, *SSs* and *ssS*) as they progress toward the sugary end of the series. The fact that the reverse is true makes it seem that the low value for the *ssS* type is presumably due to the fact that in this genotype the maternal plant (Evergreen sweet) upon which these kernels were carried was the less vigorous of the two parents, and this very likely would produce a smaller embryo and consequently a lower percentage of fat comparatively.

NITROGEN AS PROTEIN

The protein percentages in Table 3 show that the sweet corn contributes a higher value than does the parental dent corn, there being a difference of 3.1 percent. In the F_1 hybrids (F_1 seed generation) there is a tendency for a dominance of high protein which may be associated with the vigor of heterosis in the hybrid endosperm, particularly since such high values do not reappear in the later generations. In their work with nitrogen relations in corn, Pearl and Bartlett (1911) found that the F_1 generation (consisting of only one sample and made only in one direction, *SSs*) showed a dominance of the low nitrogen. Also their F_2 analyses were

TABLE 3. FAT AND PROTEIN PERCENTAGES¹.

	Endosperm genotype	Fat		Protein	
		Whole kernel	Embryo removed	Whole kernel	Embryo removed
High Fat	<i>SSS</i>	9.4	0.9	11.2	9.2
Evergreen	<i>sss</i>	8.0	1.7	14.3	12.1
F_1 (HF x E)	<i>SSs</i>	8.4	0.7	15.7	12.8
F_1 (E x HF)	<i>ssS</i>	6.3		14.1	
F_1 x E	<i>SSs</i>	7.7	0.8	11.9	10.4
	<i>sss</i>	10.8	3.1	12.5	10.7
E x F_1	<i>ssS</i>	6.5	0.4	12.8	10.7
	<i>sss</i>	9.1	2.2	13.1	11.9
F_2 starchy	<i>S</i>	7.5	0.6	10.6	13.0
sugary	<i>sss</i>	11.3	1.7	11.2	14.1

¹The same number of samples as were shown in Table 2 are used for each of the endosperm genotypes.

higher on the average than the F_1 . In the work discussed herein the F_2 protein values are distinctly lower than those in the F_1 generation. These differences may be accounted for on the basis of differential vigor of the F_1 and F_2 seeds and plants.

There is a consistent inclination for the sweet kernels to retain the higher protein values, although in none of these classes does the value attain that of the original plant nor are the differences as great as those between the parents. Presumably nitrogen content is dependent upon more than the simple genetic mechanism of the carbohydrate characters, although there is some association between carbohydrate and protein values. This conclusion does not support the statement of Pearl and Bartlett (1911) "that nitrogen content appears to behave in inheritance as an independent unit character."

PENTOSE

The parental varieties differed noticeably in pentose content, there being a difference of 3.5 percent, the sweet corn possessing the higher value. In the embryo-less material, the difference was more marked, being 4.1 percent. The F_1 seed generations exhibit an intermediate condition with a tendency toward a slight dominance of low pentose content. (Table 4).

In the F_2 and backcross generations there is an appreciable segregation of high and low pentose values associated with the starchy and sugary ker-

TABLE 4. PENTOSE AND ASH PERCENTAGES.

	Endosperm genotype	Pentose		Ash	
		Whole kernel	Embryo removed	Whole kernel	Embryo removed
High Fat	SSS	6.7	4.9	1.5	1.6
Evergreen	sss	10.2	9.0	1.8	1.7
F_1 (HF x E)	SSs	7.1	5.3	1.8	1.7
F_1 (E x HF)	ssS	7.3		1.7	
F_1 x E	SSs	7.6	4.9	1.6	1.9
	sss	9.5	7.3	1.9	1.6
E x F_1	ssS	7.1	5.7	1.7	2.0
	sss	8.0	8.3	1.9	1.7
F_2 starchy	S	6.6	5.9	1.6	2.0
sugary	sss	8.5	7.8	1.9	1.7

nel types. The differences between these phenotypes are in all cases smaller than the original parental difference, indicating that while pentose follows closely the simple endosperm factor segregation, it is independent of it to some extent. In this respect pentose differs from the total sugar, dextrin and starch situation where the differences in F_2 and backcrosses were as great or greater than the parental difference. The differences in the pentose percentages illustrating this point are as follows:

	Whole kernel	Embryo removed
Parental difference	3.5	4.1
F_2 generation difference (starchy vs. sugary)	1.9	1.9
Backcross difference— F_1 x E	1.9	2.4
Backcross difference—E x F_1	0.9	2.6

ASH

The ash percentages given in Table 4 exhibit no marked differences between parental and progeny endosperms. On the segregating ears of the hybrid generations there is a tendency for the sweet corn to carry a slightly higher ash content in the entire kernel, whereas the reverse is consistently true in the germless lot. One may deduce from this that, as far as the endosperm (and the pericarp) tissue itself is concerned, the starchy type carries a slightly greater amount of inorganic matter than does the sugary endosperm when the two types being compared are borne on the same ear. (Considering the ash analyses from the entire kernel in Table 4, there seems to be a distinct although small difference maintained between the starchy and sugary types in all of the hybrid generations, a difference that is as great as the parental difference and in the same direction (higher ash in the sweet corn). This would seem to indicate that ash content is closely associated with the carbohydrate segregation, due probably to the chemical nature of the seed tissue and not directly to any genetic determination of simple genes for ash development.

SUMMARY

Chemical analyses for total sugars, dextrin, starch, pentose, fat, nitrogen and ash were made on a series of corn kernels from crosses of Evergreen Sweet by Illinois High Fat (white dent) corn. In one lot, the whole kernels were used for analysis, in the other the embryo was removed and only the endosperm (plus pericarp) was analyzed. Parental, F_1 , F_2 and backcross generation material was analyzed chemically. In most of the material, environmental agencies were minimized because two kernel types to be compared were grown on the same cob.

The carbohydrate characters, total sugars, dextrin and starch, in which the parental varieties differed markedly, were demonstrated to have a constant interrelationship. A balanced proportion of these endosperm constituents was maintained throughout the various hybrid generations, making it certain that this interrelationship was determined by the simple, monohybrid, Mendelian mechanism (starchy-sugary genes). A cumulative effect of the endosperm genes was demonstrated quantitatively, there being a difference between the SSs and ssS endosperm genotypes.

It was also noted that the recovered sweet corn in F_2 or backcross generations possessed as high a carbohydrate index (ratio of water-soluble to water-insoluble carbohydrates) as did the original, parental sweet corn. The sugar and dextrin percentages in these sweet segregates were equally as high as those of the parental variety.

Whereas the parental dent variety (Illinois High Fat) contributed the higher fat percentage, there was a distinct reversal of fat values in the F_2 and backcross generations. In these the sweet corn segregates consistently possessed the higher fat value. Not only that, but these sweet corn kernels were recovered with a percentage of fat higher in all cases than the higher parental strain.

Nitrogen (as protein) showed but small differences in the parental and later generations. There was a consistent tendency for the sweet corn segregates both in F_2 and backcross generations to retain the higher nitrogen content, but in no case was the difference between starchy and

sugary kernels in their nitrogen content as great as the parental difference. This indicates that nitrogen formation is independent to some extent of the major carbohydrate relations.

While the pentose values of the two parental strains were different, and while there was evidence of segregation in pentose content, the differences between starchy and sugary kernel types in F_2 and backcross generations were not as great as those of the parents. This indicates that pentose content, while closely associated with the other carbohydrates, is somewhat independent of their distribution and their heredity.

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INFLUENCE OF SODIUM CHLORIDE, SODIUM CARBONATE, AND TRI-SODIUM PHOSPHATE ON GERMICIDAL EFFICIENCY OF SODIUM HYDROXIDE

MAX LEVINE, J. H. BUCHANAN and J. H. TOULOUSE

*From Departments of Chemistry and Bacteriology, Iowa State College, Ames, Iowa.**

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Many of the commercial washing compounds consist of caustic soda as a base, to which is added some other alkali, as the carbonate or phosphate of soda. It was the purpose of the following experiments to ascertain whether the addition of these milder alkalies, or of neutral salts such as sodium and potassium chloride, influences the germicidal efficiency of the sodium hydroxide.

The details of preparation of the test culture and technique of disinfection have been previously described** in a paper on the effect of concentration and temperature on the germicidal efficiency of sodium hydroxide. The time required to effect reductions of 99.9% of the exposed bacteria was employed as a basis for comparison.

Preliminary observations showed the following:

1. That addition of 1.16% NaCl, 1.5% KCl, or 1.07% Na_2CO_3 decreased the killing time of 0.5N NaOH at 50° C.
2. That NaCl KCl, Na_2CO_3 and $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ were but weakly germicidal for the test organism showing reductions of less than 30% in an hour at 60° C.

EXPERIMENTAL

OBSERVATIONS AT 50° C.

To 100 c.c. of 0.5N sodium hydroxide in a three necked Woulff bottle was added the desired quantity of sodium chloride or carbonate (dry salt) and the mixture sterilized in the autoclave at 15 lbs. for 15 minutes. After cooling, the contents of the bottle were brought to the desired temperature in a water bath, inoculated with the test organism and the surviving bacteria determined as previously described.

The results obtained at 50° C. with 0.5N sodium hydroxide to which were added various concentrations of sodium chloride or carbonate are shown in Table 1 and summarized in Table 2. The logarithms of the aver-

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age per cent surviving bacteria plotted against time are shown in Figures 1 and 2.

TABLE 1. SHOWING EFFECT OF ADDED SALTS ON SURVIVING BACTERIA IN 0.5N SODIUM HYDROXIDE AT 50° C.

Material	0.5N NaOH			
Expt. No.	82	86	91	136
Date (1926)	8-9	8-12	8-13	9-3
Time in minutes	Surviving bacteria in 5.0 c.c.			
0	943,000	696,000	971,000	985,000
10	438,000	327,000	518,000	357,000
15	343,000	255,000	399,000	263,000
20	315,000	201,000	255,000	207,000
25	161,000	148,000	144,000	151,000
30	90,000	41,300	22,300	70,000
35	16,300	4,700	1,800	-----
40	3,200	<100	<100	2,580
45	0	0	0	200
50	0	0	0	0

Material	0.5N NaOH + 1.0% NaCl		0.5N NaOH + 2.0% NaCl			0.5N NaOH + 3.0% NaCl	
Expt.No.	84	137	85	87	140	88	143
Date (1926)	8-9	9-3	8-9	8-12	9-3	8-12	9-3
Time in min.	Surviving bacteria in 5 c.c.						
0	943,000	985,000	943,000	696,000	985,000	696,000	985,000
10	438,000	315,000	403,000	254,000	278,000	263,000	280,000
15	343,000	252,000	243,000	197,000	225,000	156,000	225,000
20	221,000	154,000	88,300	81,500	109,000	26,800	11,200
25	84,000	62,000	8,400	6,100	24,300	350	975
30	7,500	8,750	880	750	1,300	0	0
35	1,150	650	250	<100	175	0	0
40	200	<100	0	0	0	0	0
45	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0

Material	0.5N NaOH + 1.0% Na ₂ CO ₃		0.5N NaOH + 2.0% Na ₂ CO ₃		0.5N NaOH + 3.0% Na ₂ CO ₃	
Expt. No.	92	138	93	141	94	144
Date (1926)	8-13	9-3	8-13	9-3	8-13	9-3
Time in minutes	Surviving bacteria in 5 c.c.					
0	971,000	985,000	971,000	985,000	971,000	985,000
10	455,000	336,000	368,000	235,000	350,000	290,000
15	290,000	245,000	298,000	195,000	242,000	215,000
20	159,000	195,000	112,000	116,000	23,600	22,300
25	45,800	107,000	5,950	34,400	1,600	1,400
30	1,050	12,300	350	1,750	<100	<100
35	0	1,400	0	200	0	0
40	0	175	0	0	0	0
45	0	0	0	0	0	0
50	0	0	0	0	0	0

Fig.1 Effect of NaCl on Germicidal Efficiency of NaOH at 50°C.

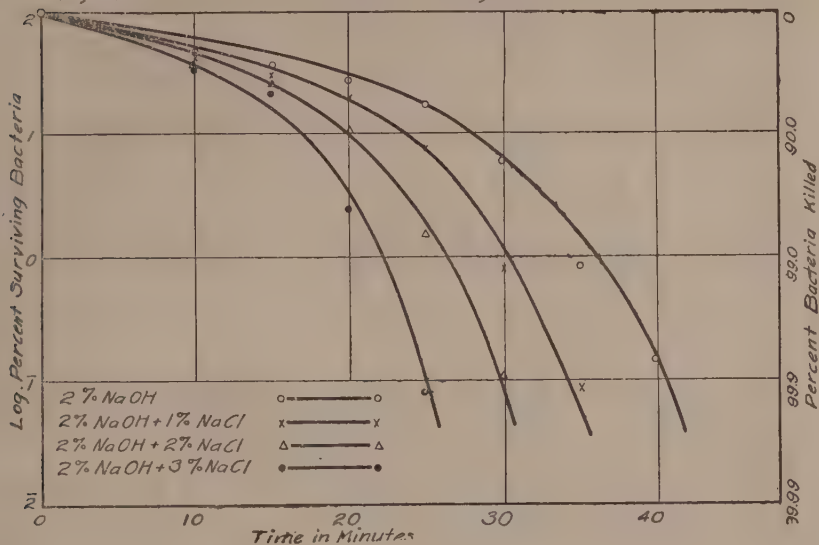
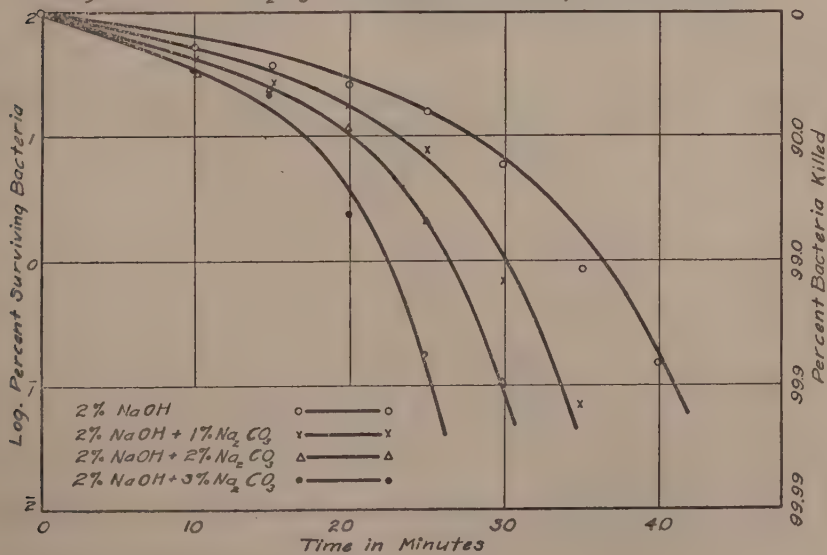
Fig.2 Effect of Na_2CO_3 on Germicidal Efficiency of NaOH at 50°C.

TABLE 1. (Continued.)

Material	0.5N NaOH with						
	*	1% NaCl	2% NaCl	3% NaCl	1% Na ₂ CO ₃	2% Na ₂ CO ₃	3% Na ₂ CO ₃
Time in minutes	Average % surviving bacteria						
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
10	45.8	39.2	35.7	33.1	40.5	30.9	32.8
15	35.5	31.0	25.6	22.6	27.4	25.2	23.3
20	27.4	19.6	10.7	2.5	18.3	11.7	2.35
25	17.2	7.61	1.54	0.08	7.81	2.06	0.16
30	6.22	0.84	0.11	-----	0.68	0.11	
35	0.87	0.09	-----	-----	0.07		
40	0.15	-----	-----	-----			

*No added salt.

It will be observed from Table 2 that the addition of sodium chloride or sodium carbonate decreased the killing time of sodium hydroxide and that the decrease was proportional to the quantities of added salts. Sodium chloride and carbonate were equally effective in this respect.

The time required to effect a reduction of 99.9% of the exposed bacteria by 2% sodium hydroxide was 41 minutes. The addition of 1, 2 or 3% sodium chloride or carbonate reduced the killing time to 34.1 minutes, 29.9 minutes and 25.3 minutes, corresponding to reductions of 16.8%, 27.0% and 38.3%, respectively.

No significant change was observed in the H⁺ ion concentration of 2.0% sodium hydroxide on addition of 1 to 3% of the chloride or carbonate.

TABLE 2. EFFECT OF CONCENTRATION OF ADDED SALTS ON KILLING TIME OF 0.5N SODIUM HYDROXIDE AT 50° C.

% added salt	Killing times for Sodium Hydroxide with	
	NaCl	Na ₂ CO ₃
0	41.0	41.0
1	34.4	33.8
2	29.9	29.9
3	25.2	25.5

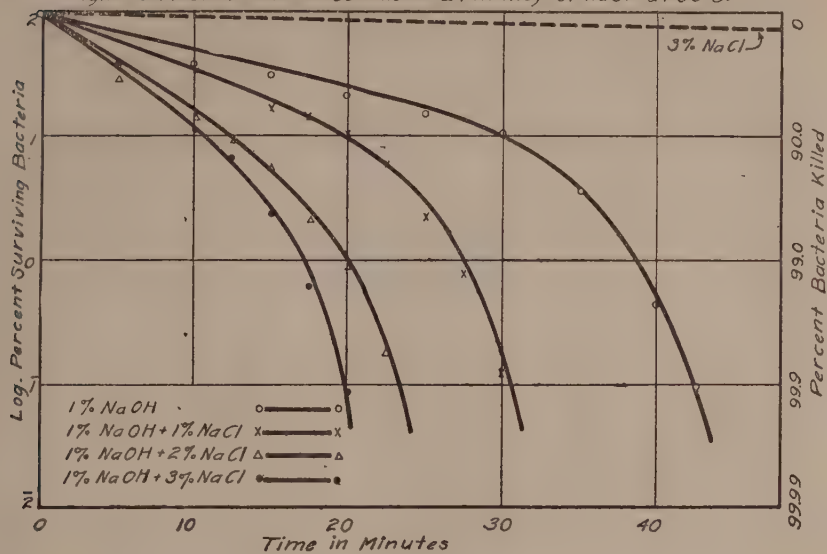
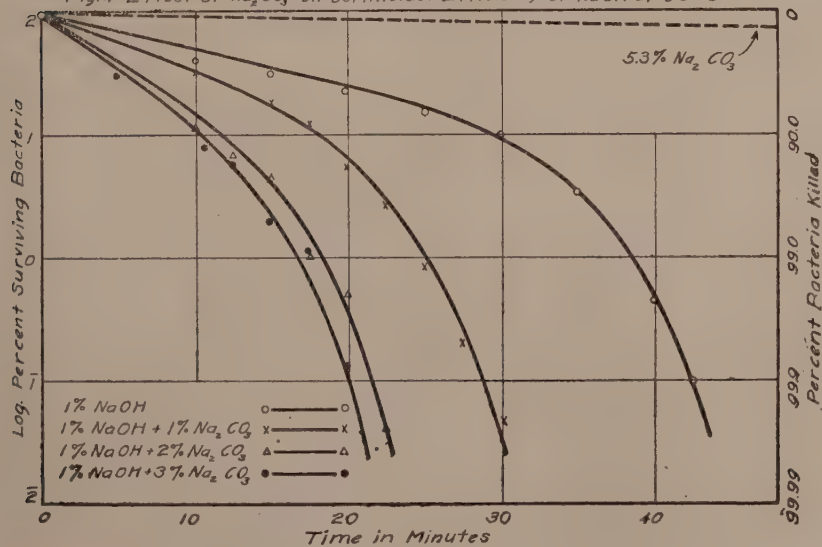
OBSERVATIONS AT 60° C.

The technique was identical with that previously described. The concentration of sodium hydroxide employed at 60° was 0.25N (or 1.0%), and sodium chloride, carbonate or phosphate were each added in concentrations of 1.0, 2.0, and 3.0%.

The results are detailed in Table 3, summarized in Table 4, and shown graphically in Figures 3, 4, and 5.

It is evident that the addition of the chloride, carbonate or phosphate distinctly decreased the killing times. The influence of sodium chloride and carbonate was approximately the same, while the phosphate was less efficient. Thus, on the addition of 1.0% NaCl, Na₂CO₃, or Na₃PO₄ 12H₂O (1.0 gram per 100 c.c. NaOH) to 1.0% NaOH, a killing time of 42.5 min-

Fig.3 Effect of NaCl on Germicidal Efficiency of NaOH at 60°C.

Fig.4 Effect of Na_2CO_3 on Germicidal Efficiency of NaOH at 60°C.

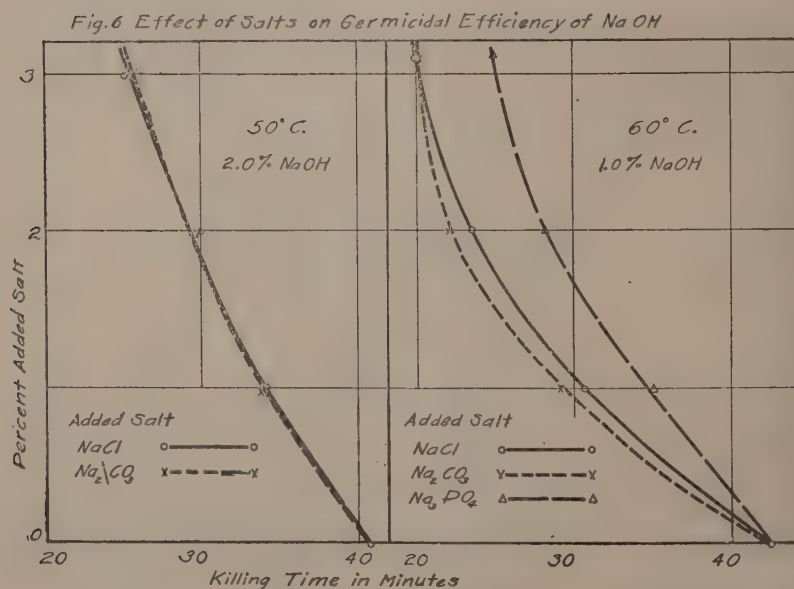
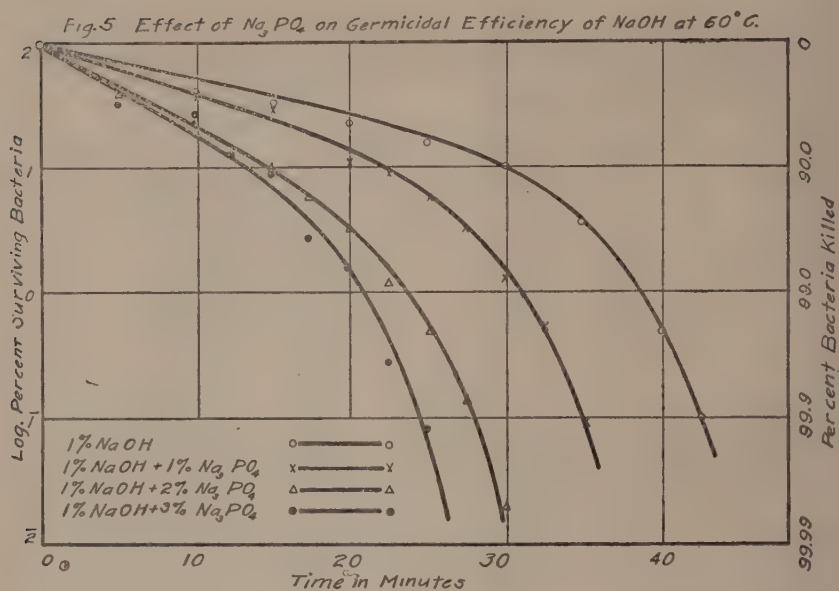


TABLE 3. EFFECT OF ADDED SALTS ON SURVIVING BACTERIA IN 0.25N SODIUM HYDROXIDE AT 60° C.

Material	0.25N NaOH		0.25N NaOH + 1.0% NaCl		0.25N NaOH + 2.0% NaCl		0.25N NaOH + 3.0% NaCl	
Expt. No.	109	120	126	127	130	133	134	133
Date(1926)	8-23	8-27	8-31	8-31	8-23	8-31	8-23	8-31
Time in minutes	Surviving Bacteria in 5.0 c.c.							
0	1,030,000	1,100,000	1,020,000	1,020,000	1,030,000	1,020,000	1,030,000	1,020,000
5	340,000	469,000	403,000	368,000	115,000	284,000	129,000	298,000
10	298,000	394,000	341,000	162,000	73,000	176,000	103,000	103,000
12.5				141,000		95,500	71,000	71,000
15						37,000	30,400	30,400
17.5						21,800	6,100	6,100
20	270,000	275,000	139,000	100,000	6,050	10,700	850	980
22.5				60,000		1,850	0	0
25	187,000	173,000	130,000	20,400	200	100	0	0
27.5				8,030		0	0	0
30	131,000	94,000	117,000	1,350	0	0	0	0
32.5				125		0	0	0
35	28,000	27,700	70,500	0	0	0		
40	4,300	5,630	4,750	0				
42.5			980					
45	375	1,450	125	0				
50	0	100	0	0				

TABLE 3. (Continued)

Material	0.25N NaOH with									
	*	1.0% NaCl	2.0% NaCl	3.0% NaCl	1.0% Na ₂ CO ₃	2.0% Na ₂ CO ₃	3.0% Na ₂ CO ₃	1.0% Na ₂ PO ₄	2.0% Na ₂ PO ₄	3.0% Na ₂ PO ₄
Time in minutes	Average % Surviving Bacteria									
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
5	38.4	33.5	27.8	29.2	31.9	11.0	30.6	37.8	32.8	32.8
10	12.5	9.36	14.3	11.3	6.96	6.72	6.84	23.0	27.4	27.4
15	32.7	16.8	5.36	2.44	18.4	4.35	6.13	29.0	13.8	13.8
17.5	21.6	13.9	2.14	0.60	12.6	1.03	1.92	10.1	9.15	9.15
20	15.6	10.9	0.82	0.09	5.70	0.50	1.08	6.42	2.59	2.59
22.5	15.6	5.89	0.18	0.01	2.64	0.04	0.13	3.18	1.62	1.62
25	10.9	2.30	0.01	0.01	0.85	0.04	0.13	1.19	0.27	0.27
27.5	10.9	0.79	0.01	0.01	0.20	0.04	0.13	0.48	0.08	0.08
30	10.9	0.13	0.01	0.01	0.05	0.04	0.13	0.14	0.02	0.02
32.5	4.05	0.01	0.01	0.01	0.01	0.01	0.01	0.55	0.09	0.09
35	0.46	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
40	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
42.5	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

*No added salt.

utes was reduced to 30.6, 29.0, and 34.9 minutes, respectively. These corresponded to reductions of 28, 30 and 17%, respectively, in the time required to effect a reduction of 99.9% of the exposed bacteria. It should be noted that these observations were made on the basis of equal quantities of the salts by weight. If equivalent quantities of the chloride, carbonate and phosphate were added on the basis of the sodium ion, the phosphate would be found to be more effective with respect to reducing the killing time.

Increasing the concentration of the added salts further reduced the killing time, but at a decreasing rate.

In the range around pH13.0 it is extremely difficult to accurately determine the H^+ ion concentration. The addition of NaCl, Na_2CO_3 , and Na_3PO_4 , in the quantities employed, to 1.0 or 2.0% NaOH was not found to be sufficient to change the H^+ ion concentration beyond the limits of error of our determinations for the hydroxide alone.

The sodium chloride and carbonate had relatively little germicidal effect on the test organism at 60° C. In concentrations of 3 to 5% of these salts there were reductions of only 30% in an hour. The sodium phosphate alone was not observed at 60° C., but at 70° C. an approximately 1.0% solution required 270 minutes to kill 99.9% of the test organism. Observations with sodium hydroxide* showed that a decrease of 10° C. increased the killing time about 3.5 to 4 times. It is, therefore, evident that the phosphate of itself was not efficient at the temperature employed.

TABLE 4. EFFECT OF CONCENTRATION OF ADDED SALTS ON KILLING TIME OF 1.0% SODIUM HYDROXIDE AT 60° C.

% added salt	NaOH with		
	NaCl	Na_2CO_3	Na_3PO_4 , 12 H_2O
Killing time in minutes			
0	42.5	42.5	42.5
1	30.6	29.0	34.9
2	23.4	21.9	28.1
3	19.9	20.1	24.7

DISCUSSION

No adequate explanation of the influence of the added salts is as yet demonstrable. It is conceivable that the undisassociated sodium hydroxide rather than the OH^- ion may be the agent which penetrates the bacterial cell then causing death. Addition of the various salts employed would tend to decrease the disassociation of NaOH and thus increase the concentration of undissociated NaOH, thereby resulting in an increased germicidal efficiency.

Another plausible explanation would be that the added salts decrease the solubility of the NaOH, in the water phase of the bacterial suspension, and consequently the sodium hydroxide is forced into the bacterial phase.

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The results summarized in Tables 2 and 4 are shown graphically in Figure 6, from which the relative effects of addition of NaCl, Na_2CO_3 and Na_3PO_4 on the germicidal efficiency of NaOH may readily be ascertained.

SUMMARY

1. The addition of sodium chloride, sodium carbonate or tri-sodium phosphate to sodium hydroxide markedly decreased the killing times at 50° and 60° C.

2. The effects of equal weights of sodium chloride and carbonate were approximately the same, whereas, the phosphate was less efficient.

3. At 60° C. a killing time of 42.5 minutes for 1.0% sodium hydroxide was reduced to 30.6, 29.0 and 34.9 minutes by the addition of 1% sodium chloride, carbonate or phosphate, respectively. In view of the fact that the chloride, carbonate and phosphate by themselves exerted but little disinfecting action, their presence with the hydroxide served to enhance the germicidal efficiency of the latter.

4. Addition of larger quantities of these salts further decreased the killing times of sodium hydroxide, but at a decreasing rate.

5. It is suggested that the undissociated sodium hydroxide may be the agent which penetrates the cell. The addition of the various salts would increase the concentration of undissociated sodium hydroxide, or possibly decrease the solubility of the hydroxide in the water phase which would tend to force it into the bacterial phase of the suspension. In either case the effect would be to increase the death rate of the bacteria.

A STUDY OF THE PREPARATION OF SIRUPS*

I. *Relation of Temperature to Concentration of Sirup and Alkali on the Viability of Yeast Spores***

E. E. PETERSON with MAX LEVINE and J. H. BUCHANAN

From the Departments of Chemistry and Bacteriology, Iowa State College.

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One of the most important problems confronting the bottled carbonated beverage industry is the production of a beverage which will not deteriorate or spoil even when this product is stored for long periods of time. If such a beverage is to be prepared, either (1) the material entering into its composition must be sterile and handled aseptically, or (2) conditions within the bottle must be such that there will be no growth of micro-organisms which may have been present in any of the materials going to make up the finished product.

Of the 734 samples of spoiled carbonated beverages examined in 1925-26, 83.3% were found to contain yeast. In many cases even casual examination showed flocculent sediments which, when examined microscopically, were found to consist principally of yeast cells.

It was quite evident that yeasts were the primary cause of spoilage. Examination of numerous samples of water (both raw and carbonated), simple and flavored sirups, and the finished products from establishments which were encountering spoilage difficulties, showed that the sirups constituted the primary source of yeast contamination. Experiences with our experimental bottling plant indicated that sterilization of sirup-making equipment was not sufficient to eliminate yeast spoilage, and attention was therefore directed to the raw material—sugar.

Accordingly, sterile sample bottles with instructions for proper sampling were sent to 125 representative manufacturers of carbonated beverages in 28 states. These manufacturers had previously expressed a willingness to cooperate on this problem. Of the 132 samples of sugar obtained, 62 (47%) were found to contain yeast in 5 gram portions.¹

Since the prevalent method for preparing sirup is the cold process, conditions are usually suitable for the survival of yeasts which may be present in the sugar and their subsequent growth in the finished beverage. Owen says that the process of refining sugar does not leave the sugar sterile and that there are organisms capable of withstanding the temperature of the processes and which survive in the form of spores.

An investigation of the resistance of these spores of the species found in sugar showed that they could survive a 3 to 4 hour exposure to stream-

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**These studies were made possible through a Fellowship maintained by the American Bottlers of Carbonated Beverages at Iowa State College.

ing steam. Owen³ also showed that this sugar when inoculated into sterile 10% sirup yields gum-forming species of bacteria almost exclusively. When grown in 25% sugar solution, unequal numbers of yeasts and bacteria appear with the latter predominating. In 50% sugar solutions, a mixed growth of yeasts and bacteria is also obtained, but the yeasts are predominating. Owen makes the following statement with regard to less highly refined sugars—"That the sugars used in these experiments were not abnormal in respect to their mixed flora of bacteria and yeast appears evident from a direct microscopical examination of about 70 samples of different qualities of raw sugars, about 40% of which were found to contain yeast cells." Dubourg⁴ reported a yeast which was still active at a concentration of 80% sugar. Owen⁵ isolated *Saccharomyces zopfii* from heavy sirup and characterized this species by (1) its ability to ferment highly concentrated sugar solutions and (2) its resistance to heat. McKelvey and associates have shown that yeast cells in the vegetative form would die off rather quickly in sirups of high density, but this does not hold true for spores.^{6,7} Usually, however, the sirup is not stored for any great length of time before it is diluted to make up the flavored sirup, which subsequently is diluted again upon being bottled. Thus the yeast cells or spores which may have been originally present in the dry sugar find conditions favorable for germination and growth.

The acid content and carbon dioxide pressure of carbonated beverages are generally effective in keeping bacterial and mold growths to a minimum or in eliminating them entirely. As far as these two agents of spoilage are concerned, it is usually possible to use enough acid or have a great enough carbon dioxide content to prevent them from growing without making the beverage objectionable because of its sourness or high carbonation. Many spoiled products having a high acidity as well as good carbonation have been found to contain yeasts. It seems improbable that the use of as great quantities of these acids as is feasible without seriously affecting the flavor and taste of the beverage, would completely eliminate this type of spoilage. Weldin⁸ reports that with agar media it took a pH range from 2.6 or 3.3 to inhibit growth of yeasts while broth media showed that a pH range from 2.3 to 2.7 was necessary to inhibit the growth of these same organisms. The reaction of carbonated beverages is rarely more acid than pH 3.0 to 3.5 (after the CO₂ has been removed). Obviously, then, some method must be found to eliminate these yeast cells and spores from the simple sirup unless the beverage manufacturer wishes to resort to the pasteurization of all of his bottled products.

Siebel⁹ contends that the original source of yeast contamination is from bottles which have not been adequately washed rather than from the sugar. Accordingly it was deemed advisable to investigate the reaction of yeast spores when subjected to the action of hot alkali solutions at temperatures generally recommended for the washing of bottles.

The preceding discussion led to the experimental consideration of the following topics:

1. Relation of the time of exposure and temperature in sterilization of sirups contaminated with yeast spores.
2. Relation of the temperature of exposure, concentration of alkali, and period of exposure to the death rate of yeast spores.

A. *Viability in Sirups.*

All of the sirups used in this series of experiments were prepared from a highly refined sugar whose normal solution gave a reading of 99.9 in a 200 mm. tube on a saccharimeter. The moisture content of this sugar was 0.028%. These sirups were made up on the density basis by using the proper amount of distilled water to furnish the density of sirup desired. Three densities of sirup were used in this series—24° Baumé, 30° Baumé, and 36° Baumé. These are equivalent to specific gravities at 20°C/20°C of 1.1984, 1.2609, and 1.3303, respectively.

The spores were prepared from cultures of spore-forming yeasts which had been isolated from spoiled carbonated beverages¹⁰. A special medium of carrot agar was used as this was found to be especially suitable for the formation of spores by yeast.¹ This carrot calcium sulfate agar was prepared by grinding up about four pounds of carrots (saving the juice) and extracting the pulp with about two liters of boiling distilled water. The total volume of juice and extraction water was two liters. This liquid was saturated with calcium sulfate and 2% of agar agar was added. This was autoclaved at 15 uponds steam pressure for 20 minutes.

About 50 cubic centimeter portions of this medium were transferred to each of 28 Kollé flasks. These were then rather heavily plugged with cotton and autoclaved for another 20 minutes. Slants of carrot agar were made in 28 test tubes at the same time. After cooling and solidification of the agar had taken place, the Kollé flasks were inoculated evenly over the surface by the use of sterile swabs. One Kollé flask was used for each of the 28 cultures of the spore-forming yeasts. The slants of carrot agar were also inoculated with the same cultures. The Kollé flasks and tubes were incubated for 17 days, when numerous spores were demonstrated by making spore stains of the cultures which were grown in the tubes. The yeast growths on the surface of the Kollé flasks were scraped off by means of a hoe-shape Monel metal tool which had previously been sterilized in a flame. The scrapings were all mixed together in a sterile evaporating dish and dried at 45°C in a vacuum oven. After drying, the spores were ground up in an agate mortar under aseptic conditions and screened to pass a 200 mesh sieve. The dry weight of the mixture of the 28 yeast spore cultures was 3.9289 g. A count of 72,000,000 per gram was obtained by plating out a small portion. In order that the same initial contaminations might be readily obtained in this series of experiments, the spore mixture was diluted with 9 times its weight of sterile powdered lactose and thoroughly mixed.

The technique of disinfection was briefly as follows: placed 100 cubic centimeters of the sirup density desired into a 3-necked, round-bottomed Pyrex flask of 200 cubic centimeters capacity, a one-hole cork was fitted with a suitabel stirring rod and placed in the middle neck of the flask. The other two necks were plugged with cotton. This flask was then sterilized in a steam autoclave at 15 pounds of steam pressure for 20 minutes. Following this, the flask was immersed in a DeKhotinsky water bath which had previously been regulated to the proper temperature. The stirring rod was connected to a motor and set in motion. After allowing it to remain in the bath for about 30 minutes, the contents of the flask would be at the desired temperature. Then one cubic centimeter of a 10% suspension (1.0 g. in 10 c.c.) of the spore mixture was added to the sirup by means of a

capillary pipette, taking care not to contaminated the sides of the neck. At stated intervals, 5 cubic centimeters of the sirup were withdrawn with a sterile pipette and put into 45 cubic centimeters of sterile malt extract broth. Counts were obtained on wort (Difco) agar by plating duplicate one centimeter and 0.1 cubic centimeter portions of this broth, and incubating for 48 hours at 25 to 28°C.

The initial count was obtained by inoculating 100 cubic centimeters of sterile tap water with 1.0 c.c. of the yeast spore-suspension, using the same capillary pipette as was previously employed. After thorough shaking to insure an even distribution, a 5 cubic centimeter portion of this was transferred to 45 cubic centimeters of the malt extract broth. One cubic centimeter and 0.1 cubic centimeter quantities of this broth were plated out. Thus the count on the one cubic centimeter plate should represent the initial count for one cubic centimeter of the broth which had received 5 cubic centimeters of sirup, since the same dilutions had been made in both cases.

Smears were prepared and examined from colonies on the plates when the survivors numbered a dozen or less per plate. This was to insure that contamination had not occurred and that the survivors were actually yeasts. The flasks of broth were incubated for 4 days and observed at the end of 2, 3, and 4 days for turbidity and sediment. This was designed to serve as a check on the plating process. The sediment from the last turbid flask in a series was always examined to determine whether or not the turbidity was due to yeasts.

Owen¹¹ says that the sterilization temperatures to which sirups are preheated in canning vary inversely with the volume of the cans. He gives temperatures varying from 71°C. to 81°C. for Number 10 to 1½ cans, but gives no hint as to the length of time that elapses after the sealing of the can until the sirup cools down. The required temperatures vary inversely with the size of the cans because the larger cans will cool more slowly. In regard to the heat resistance of *Saccharomyces zopfii* Owen¹² says that a temperature of 90°C. for 10 minutes was insufficient to effect complete sterilization. He further adds that, while the resistance of the yeast to high temperature does not vary directly with the density of the solution in which it is grown, it does seem to retain its power of fermentation more persistently when subjected to such temperatures in thick sirup than in more dilute solutions.

McKelvey¹ states that, in general, the higher the concentration of sugar the longer was the time required to kill the yeast spores under examination.

Since Owen⁵ found a temperature of 90°C. to be insufficient for sterilization, several runs were made at 100°C. Results typical of the several runs are shown in Table I. It will be noticed that the higher the concentration of sugar in the sirups, the longer was the time required to kill the yeast spores which had been inoculated. As samples were removed in 5 cubic centimeter quantities, counts are given in terms of this unit.

Detailed data are given in Table II for the death rate of the yeast spores in the 36° Baumé sirup. From these data the curves on Plate I were constructed.

The sudden decrease in the first two minutes is explained by the fact that the spore mixture contained quite a few vegetative cells. These are

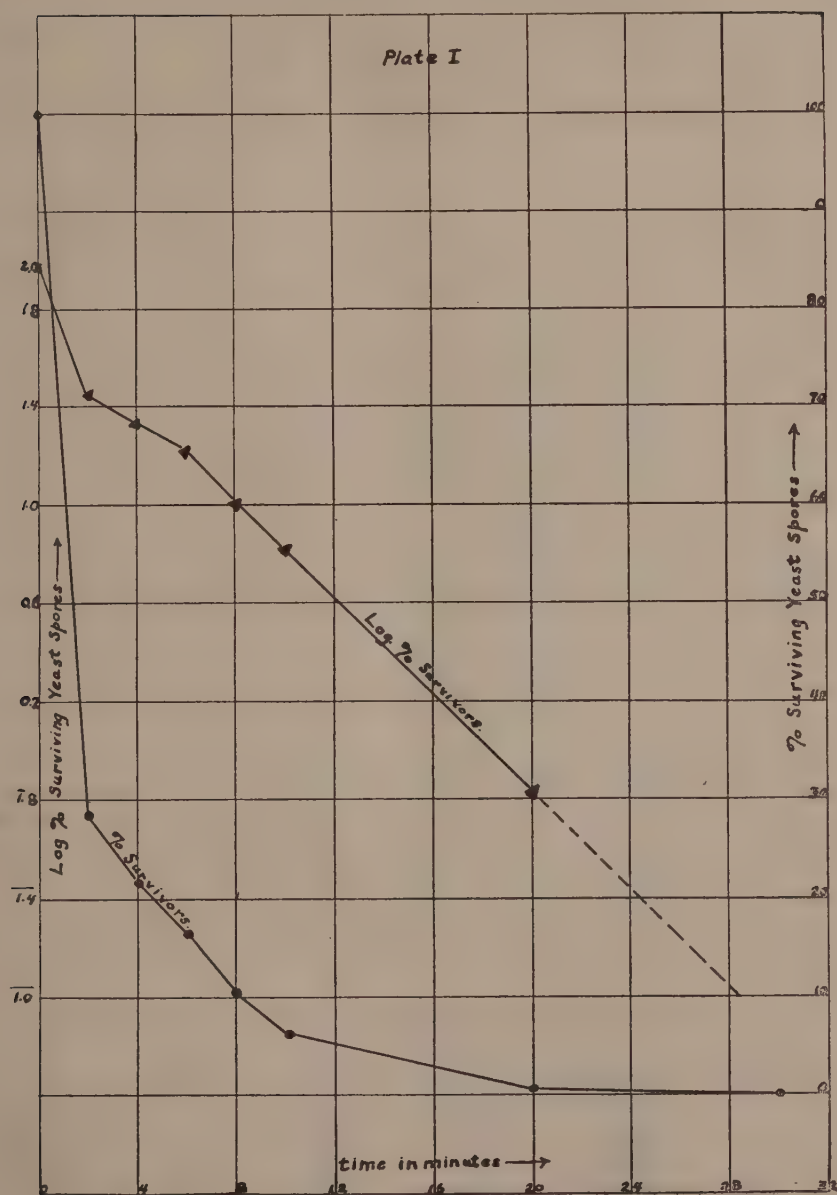


TABLE I. TIME REQUIRED TO KILL 99.9% OF YEAST SPORES IN SIRUPS AT A TEMPERATURE OF 100°C.

Density of sirup	Concentration Sucrose ex- pressed as % by weight	Time required to kill 99.9% of yeast spores
Distilled water	0	2-4 minutes
24° Baumé sirup	44.2	6 minutes
30° Baumé sirup	55.6	8-10 minutes
36° Baumé sirup	67.3	28 minutes

Inoculum 36,000 per 5 c.c. of sirup.

less resistant than the spores. After the vegetative cells have been killed, the death rate increases more or less regularly for the rest of the experiment. Extrapolation at the same rate of death between 20 and 30 minutes as was shown between 10 and 20 minutes shows 28 minutes as the time for the killing of 99.9% of the yeast spores. In general, the velocity of the death rate is a constantly increasing one after the first initial drop has occurred.

The time of killing may be a function of the amount of moisture as well as the temperature. These yeast spores apparently cannot absorb as much water from a 36° Baumé sirup (nearly a saturated sucrose solution at room temperature) as from the less concentrated sirups or distilled water. Dry sterilization invariably requires a higher temperature or a longer period of exposure than does moist sterilization. These may serve as explanations of the fact that the spores in the more concentrated sirups are more resistant to the influence of high temperatures than the spores in the less concentrated sirups.

Preliminary experiments showed that yeast spores could not be readily killed at 70°C. Exposures for 60 minutes in distilled water showed about one-third of the spores still survived. The sirups seemed to exert a slight protective action on the spores, the more concentrated sirups having a greater per cent of survivors than the less concentrated ones.

In order to reduce the time of sterilization for concentrated sucrose sirups, a small amount of acid was added to the 36° Baumé sirup. One cubic centimeter of a 7.074 normal solution of citric acid was added per

TABLE II. SHOWING SURVIVING YEAST SPORES IN 36° BAUME SIRUP AT 100°C.

Time of exposure in minutes	Counts per 5 c.c. unit of sirup run A	Counts per 5 c.c. unit of sirup run B	Average count per 5 c.c. unit of sirup	Average % survivors	Log of average % survivors
0	28,500	43,000	35,750	100.0	2.000
2	11,250	9,250	10,250	28.65	1.457
4	7,900	7,750	7,825	21.9	1.341
6	5,900	6,100	6,000	16.8	1.225
8	3,750	3,750	3,750	10.5	1.020
10	2,500	2,250	2,375	6.65	0.822
20	250	250	250	0.7	1.845
30	0	0	0	0	*****

36° Baumé sirup (Sp. Gr. at 20/20°C. = 1.3303).

100 cubic centimeters of sirup (equivalent to the manufacturer's one ounce of 50% citric acid solution per gallon of sirup). At 100°C. no growth was obtained after 2 minutes exposure. This is in contrast with the 28 minutes required to sterilize the same sirup without the addition of acid.

B. Viability in Alkali Solutions.

The preparation of the dried yeast spores was that described in the preceding section.

The solutions of alkalis used were of sodium hydroxide and of a commercial alkali consisting of a mixture of sodium hydroxide and sodium carbonate. The commercial alkali will be designated as Alkali A.

The sodium hydroxide solutions were freed of their carbonate content by being made up in a 50% stock solution. Sodium carbonate is practically insoluble in this concentration of sodium hydroxide. After cooling, the solution was filtered through a Gooch crucible, using a pad of asbestos which had previously been prepared according to the Munson-Walker method for gravimetric sugar determinations. This filtered sodium hydroxide solution was then diluted with freshly boiled distilled water until its titration value showed it to be at the concentration which was desired.

The solutions of Alkali A were made up from a stock solution of approximately 5% strength. The titration value of this was determined, using methyl orange as the indicator, and the proper dilutions were made with carbon dioxide-free water.

Table III shows the titration values of both the sodium hydroxide and Alkali A solutions. Titrations were made with 0.2519N HCl using phenolphthalein and methyl orange as indicators.

Essentially the same method of procedure was followed in this series of experiments as in the sterilization of sirups. One hundred cubic centimeters of alkali solution were substituted for the sirup. Forty-five cubic centimeters of a sterile acid solution were substituted for the 45 cubic centimeters of malt extract broth. Each flask of this acid solution contained a drop of methyl orange solution and sufficient acid to neutralize 5 cubic centimeters of the alkali solution which it was to receive. If the indicator showed that the end-point had not been reached after the addition of the 5 cubic centimeters of alkali solution, neutralization was completed with a drop or two of a sterile 1% sodium hydroxide solution. One cubic

TABLE III. TITRATION VALUES OF ALKALI SOLUTIONS.

Alkali	% Alkali calculated as NaOH	Titration values cc. 0.2519N HCl = 5 cc. alkali		G. Na ₂ CO ₃ per 100 cc. solution	G. NaOH per 100 cc. solution
		P.P.*	M.O.*		
NaOH	1.0%	4.95	5.00	0.027	0.989
NaOH	2.0%	9.90	10.00	0.053	1.975
NaOH	3.0%	14.70	14.89	0.101	2.924
NaOH	4.0%	19.65	19.90	0.134	3.909
Alkali A	1.0%	3.45	4.96	0.806	0.391
Alkali A	2.0%	6.88	9.91	1.621	0.776
Alkali A	3.0%	10.37	14.80	2.366	1.197
Alkali A	4.0%	14.10	19.93	3.113	1.667

*P.P. = phenolphthalein used as indicator.

*M.O. = methyl orange.

centimeter quantities from these flasks were plated out in duplicate, using the wort agar described in the previous section. The initial count was obtained in the same manner as previously outlined and the same precautions were taken to insure that the colonies on the plates (when only a few were left) were yeasts.

Duplicate runs were made at temperatures of 50°C., 60°C., and 70°C. At 50°C., 2%, 3%, and 4% solutions of both Alkali A and NaOH were used. At 60°C., 2%, 3%, and 4% solutions of Alkali A and 1%, 2%, and 3% solutions of sodium hydroxide were used. At 70°C., 1% and 2% solutions of Alkali A and a 1% solution of NaOH were used. Distilled water was also run for a comparison.

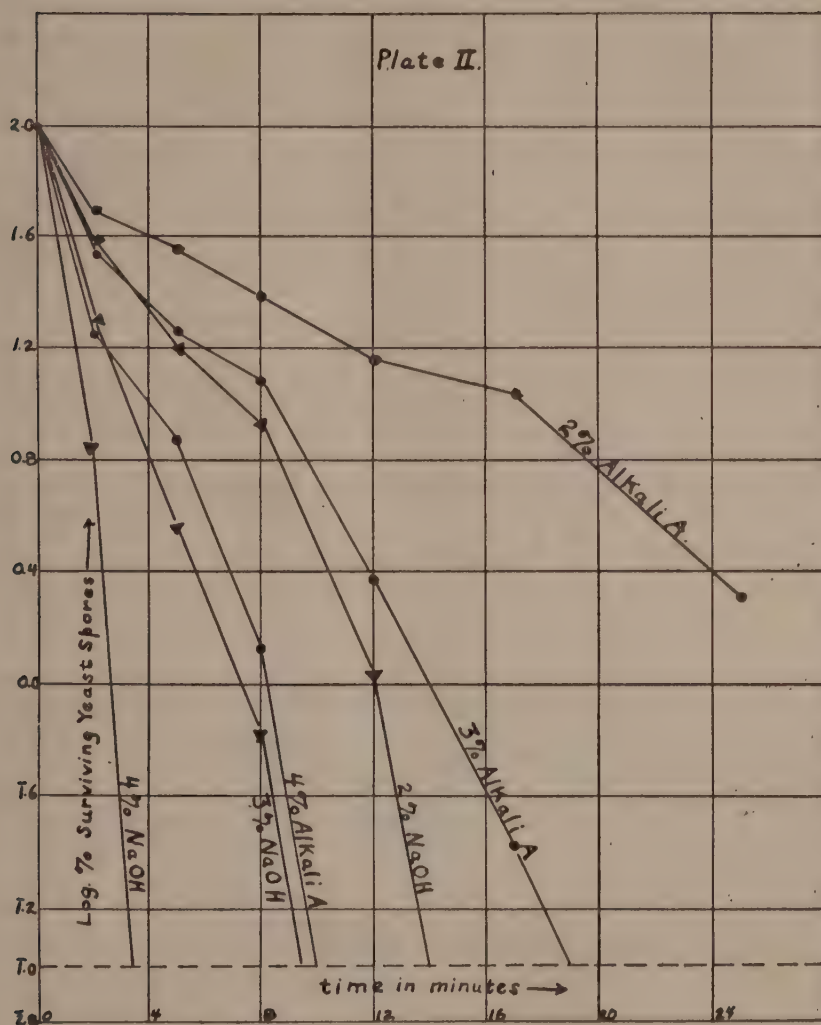
Table IV gives the time in minutes required to kill 99.9% of the yeast spores when exposed to the action of the different alkali solutions at 50°, 60° and 70°C. It will be noticed that the time required to kill 99.9% of the yeast spores may be reduced either by increasing the concentration of the alkali or by increasing the temperature. It is also apparent that it takes somewhat less than twice the concentration of Alkali A to be as effective at any given temperature as it does of the sodium hydroxide solution. In other words, the germicidal efficiency of the sodium carbonate content is less than that of an equivalent amount of sodium hydroxide. The germicidal efficiency of a sodium carbonate and sodium hydroxide mixture, however, is greater than can be accounted for by its sodium hydroxide content alone.

TABLE IV

Alkali solution	% Alkali calculated as NaOH	Temperature °C.	Time in minutes required to kill 99.9% yeast spores
NaOH	2%	50	15
NaOH	3%	50	9.5
NaOH	4%	50	3.5
Alkali A	2%	50	More than 25
Alkali A	3%	50	19
Alkali A	4%	50	10
NaOH	1%	60	9
NaOH	2%	60	3.5
NaOH	3%	60	2
Alkali A	2%	60	7
Alkali A	3%	60	4.5
Alkali A	4%	60	3
NaOH	1%	70	less than 2
Alkali A	1%	70	less than 2
Alkali A	2%	70	less than 2

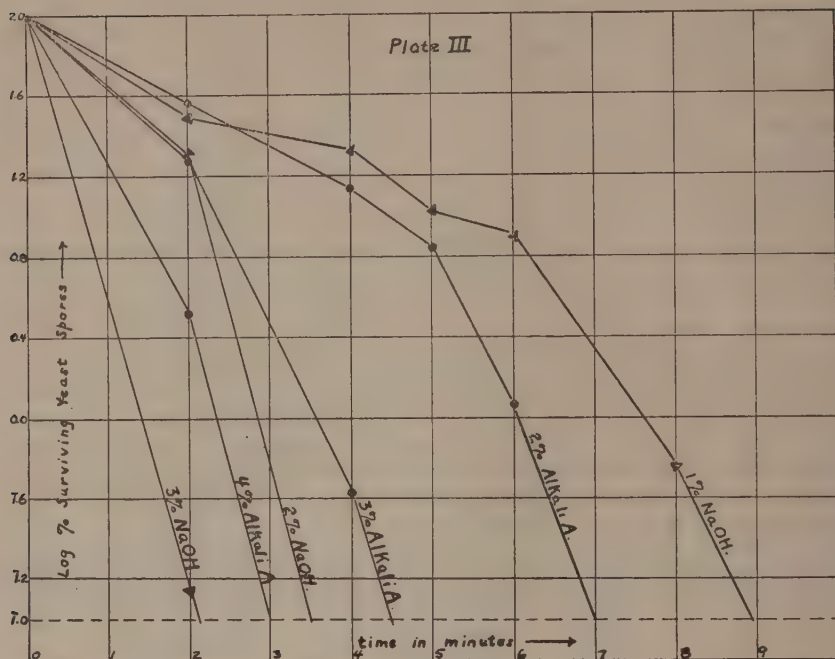
Plate II at 50°C. and Plate III at 60°C. show the rates at which the yeast spores are killed. From these curves the per cent of surviving yeast spores in any of the alkali solutions after different periods of time may be estimated. The large initial drop in the count during the first two minutes which was commented upon in Plate I is again quite apparent in Plate II, although not so noticeable in Plate III.

Levine, Buchanan and Lease¹² show data and curves for the death rate of a spore-forming bacterium. Disregarding the rapid initial drop which occurs in the first two minutes, there is a striking similarity in the general shape of the curves obtained by plotting the logarithm of the per



cent of survivors against time. Both of these series show a death rate velocity which increases as the time of exposure is increased. The rates of death clearly do not follow the rates for monomolecular chemical reactions for in that event a straight line would be obtained upon plotting the logarithm of the per cent of survivors against time. In other words, there would be a constant death rate; these data show a death rate which increases with increasing time of exposure.

The rate of killing yeast spores in distilled water at 70°C. was determined for the sake of comparison with those of the alkali solutions. Table V shows the per cent of surviving yeast spores after different lengths of time. It shows 46.2% of the spores to be surviving at the end of 30



minutes exposure to a temperature of 70°C. in distilled water. This agrees with results obtained in the sterilization of sirups where it was found that one-third to one-fourth of the number of yeast spores originally present were alive at the end of an exposure for 60 minutes at 70°C. in distilled water. The slight fluctuation in the per cent of survivors may be accounted for by the breaking up of clumps of yeast spores into smaller clumps or individual cells, when subjected to soaking combined with constant stirring.

TABLE V. SHOWING THE PER CENT OF SURVIVING YEAST SPORES IN DISTILLED WATER AT 70°C.

Time of exposure in minutes	% yeast spores surviving at 70°C. in distilled water
0	100.0
5	50.5
10	47.3
15	45.6
20	41.4
25	49.5
30	46.2

SUMMARY

1. At the temperatures observed (60-100°C.), the greater the concentration of sucrose in the sirup, the longer was the time required to kill yeast spores.

2. The time for sterilization of sirups inoculated with yeast spores may be materially reduced by the addition of acid to the sirup.

3. A temperature of 100°C. for 28 minutes was sufficient to sterilize a 36° Baumé sirup. In actual practice the sterilization may be effected by bringing the sirup to a boil and boiling for 5 minutes. If citric acid is added in the proportion of 1 c.c. of a 7.074 normal solution to 100 c.c. of sirup, sterilization may be effected by bringing the sirup to a boil.

4. Exposure to a temperature of 70°C. in distilled water for an hour was not sufficient to kill dried yeast spores.

5. The time required for killing 99.9% of the yeast spores in washing solutions may be reduced either by (a) increasing the concentration of alkali or (b) raising the temperature.

6. Sodium hydroxide at a given concentration and temperature has a greater germicidal efficiency than a chemically equivalent solution of a mixture of sodium hydroxide and carbonate at the same temperature. The germicidal efficiency of a sodium hydroxide-carbonate mixture is greater than can be accounted for by the sodium hydroxide content alone.

7. There is little likelihood of bottles being the source of yeast contamination if a 2% sodium hydroxide or a 3% alkali A solution is used in the bottle washing process at a temperature of 60°C. with a period of contact amounting to 5 minutes.

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A STUDY OF THE PREPARATION OF SIRUPS*

II. *Hydrolysis of Sucrose Sirups***

E E. PETERSON with J. H. BUCHANAN and MAX LEVINE

From the Departments of Chemistry and Bacteriology, Iowa State College.

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In addition to changes in bottled carbonated beverages caused by microorganisms, the manufacturer is concerned with a chemical change. The sucrose of beverages which are stored for any length of time undergoes a slow inversion catalyzed by the acid of the beverage. This is one of the principal changes which takes place in the aging of ginger ales¹—a practice which is said to mellow them. The inversion which occurs has also been named as the cause of “off-flavor” in beverages which were stored for a considerable period of time before being consumed.^{2, 3} The invert sugar which is produced by the decomposition of the sucrose is less sweet than the sucrose from which it is formed, different investigators evaluating its sweetness at 78 to 95 on the basis of sucrose as 100.^{4, 5, 6}

There seems to be some disagreement concerning the sweetness of levulose⁶, values having been reported ranging from 103 to 150. Spengler and Traegel⁷ assign a value of 108 for its sweetness, which is in good agreement with Paul's value⁸ of 103.3. Bates⁹ on the contrary agrees quite closely with Deerr¹⁰ and Sale and Skinner on a sweetness value near 150 for levulose. These same workers agree more closely on the sweetness value for dextrose, assigning it a sweetness of 50 to 60 on the same basis. No matter which values of sweetness are taken, an artificial invert sugar made from equal parts of dextrose and levulose would be less sweet than an equivalent quantity of sucrose. This loss of sweetness caused by inversion is sufficient to account for the change of taste of beverages upon long storage.

The hydrolysis of sucrose has been a much investigated subject, but, unfortunately, nearly all of the work has been done with dilute solutions of sucrose and at low temperatures. The primary object has been to investigate the mechanism of the reaction rather than the production of a large amount of invert sugar. Caldwell¹¹ gives a rather complete bibliography up to 1906 on the hydrolysis of sucrose.

Spencer¹² studied the effect of citric, tartaric, phosphoric, and sulfuric acids on the rate of inversion of sucrose. She gives a review of the literature up to and including 1925. Spencer worked with sucrose and acid solutions at the concentrations in which they are found in the finished beverage. She concludes that (1) in a given solution, increasing the concentration of sucrose increases the velocity of the reaction to a small ex-

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tent. (2) For tartaric, citric, and phosphoric acids the rate of reaction decreases as the reaction proceeds until a point of equilibrium is reached. This point of equilibrium depends for a given acid with a definite concentration of sucrose upon the pH of the solution. (3) For a given pH and a given concentration of sucrose the rate of reaction varies with the kind of acid, being considerably greater for phosphoric acid than for citric or tartaric acids. Tartaric acid inverts slightly faster than citric acid, but the difference is scarcely appreciable.

Many workers^{13, 14, 15, 16, 17, 18, 19} have attempted to apply the general equation for a unimolecular reaction to the hydrolysis of sucrose by using empirical formulae or by substituting activities instead of concentrations for sugar and hydrogen ion. Assumptions have also been made that the sucrose molecules were hydrated with five, six or more molecules of water. Pennycuik²⁰ studied the reaction and determined k (unimolecular). He found that k made a steady increase during the inversion process and concluded that the decrease in water content and the increase in H activity during inversion are sufficient to explain the steady increase in the value of k . Scatchard²¹ says that uncertainty as to the activity of the H ion in solutions containing sucrose makes inconclusive the attempts to determine the mechanism of the inversion process by fitting the rates of reaction quantitatively with formulae containing these activities. Even the difference between the change of liquid junction potential with flowing and with stationary junction amounts to 11% in the hydrogen activity between 0 and 700 grams of sucrose per liter, while the change in the water activity in this range is less than 9%. Obviously the number of mols of water that enter into the reaction cannot be accurately determined.

Since very few of these investigators worked with sucrose solutions concentrated enough to be called sirups and much of the foregoing work was inapplicable because it was done at such a low temperature that inversion took place only slowly, it was decided to use citric and tartaric acids in the concentrations usually employed in flavored sirups and to use a temperature near to the boiling point of the sirup.

The sucrose used in this series of experiments was a highly refined cane sugar with a moisture content of 0.028%. A solution of 26 grams of this sugar, when made up to 100 cubic centimeters volume with distilled water, gave a reading of 99.9 on the saccharimeter (200 mm. tube) at 20°C.

The citric and tartaric acids used were of the C.P. grade. No further purification was made. A 7.074 normal citric acid solution and a 6.553 normal tartaric acid solution were made up and used since the addition of one cubic centimeter of these acid solutions to 100 cubic centimeters of sirup would maintain the same ratio of acid to sugar as the manufacturer of carbonated beverages uses in preparing his flavored sirups—(one ounce of 50% citric or tartaric acid solution per gallon of sirup).

A small constant temperature oil-bath was made from a hot-plate, asbestos boards, a six quart aluminum pan, and a DeKhotinsky bimetallic electric thermo-regulator. A fluctuation from the temperatures desired of 0.2°C. was observed as the maximum divergence. More frequently the temperature was maintained with a fluctuation of 0.1°C. or less.

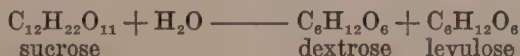
Sirups were prepared by weighing the proper amount of sucrose required for the particular sirup desired, transferring this to a calibrated volumetric flask and making up to volume at 20°C. with distilled water.

Heat was required for the complete solution of the sucrose in the more concentrated sirups.

Two hundred cubic centimeters of sirup were transferred to a 3-necked, 400 c.c. round-bottomed Pyrex flask, which was fitted with a motor stirring device through the middle neck. One of the other necks was fitted with a thermometer and cork, while the other neck was closed with a cork. The flask was immersed in the bath until it had reached the desired temperature. Then the acid was added with a pipette through the third neck of the flask. The motor stirrer functioned continuously throughout each experiment. At stated intervals, a quantity of sirup was withdrawn from the reaction flask and transferred to calibrated 50 c.c. volumetric flasks which contained enough sodium hydroxide solution to neutralize the acid which was contained in the sirup. The transfer pipette was rinsed each time with distilled water into the volumetric flask in order to eliminate the factor of varying amounts of drainage due to different densities of sirups. The transfer pipette which was used in all of the hydrolysis experiments was found to contain 9.933 cubic centimeters of liquid when calibrated at 100°C.

The 50 c.c. flasks which contained sufficient sodium hydroxide solution to neutralize the acid were placed in a pan of cracked ice so that the temperature might be reduced suddenly to further aid in stopping inversion. The contents of the flasks were made up to volume at 20°C. and polarized in a 200 mm. tube using a Schmidt and Haensch single-wedge saccharimeter. The saccharimeter tube was a jacketed one, fitted with a thermometer so that the temperature of the solution under examination was known at all times. All saccharimeter readings were made at 20°C. The initial reading was obtained by withdrawing some sirup which had been brought up to temperature in a separate container in the same oil bath. This was transferred to a 50 c.c. volumetric flask to which had previously been added equivalent quantities of sodium hydroxide and citric or tartaric acid in amounts corresponding with the other flasks of the experiment. This was to compensate for any effect which the sodium citrate or tartrate might have on the polarization.²²

The results of the experiments are given in the following tables numbers I to XII inclusive. Values of k were obtained by the use of equations based on Guldberg and Waage's law of mass action, which states that the reaction velocity, or rate of change of concentration, must be proportional to the concentration of each of the reacting substances. The reaction which takes place upon the hydrolysis of sucrose is the formation of one molecule of dextrose and one molecule of levulose for each molecule of sucrose decomposed. The equation



empirically expresses the change which occurs. It will be seen that a molecule of water enters into the reaction for each molecule of sucrose decomposed. Because the concentration of water changes so little compared to that of the sucrose the assumption is usually made that the concentration of water is constant (at least for dilute solutions). The k for a unimolecu-

lar reaction is customarily used to express the rate of reaction for sucrose inversion. Using the Briggsian logarithms the equation is

$$k = \frac{2.3026}{t} \log \frac{a}{(a-x)}$$

where "t" is the time in minutes and "a" is the initial concentration of sucrose and "x" is the concentration changed at time "t". Very close agreement in the values of "k" at different times throughout the course of the reaction is not to be expected since the simplifying assumption, that the water does not appreciably change in concentration, is in error when the equation is applied to concentrated solutions.

The per cent of sucrose remaining unchanged at time "t" was calculated as follows:

$$\% \text{ sucrose} = \frac{\text{reading at time "t"—final reading}}{\text{total change in reading}} \times 100$$

When numerical values of the saccharimeter readings at any time are substituted in the above equation, it yields a value for (a-x) or the per cent sucrose remaining unchanged. The initial concentration of sucrose, "a", may be used by taking 100 as its numerical value. The equation for calculating "k" then becomes

$$k = \frac{2.3026}{t} (2.000 - \log \% \text{ sucrose})$$

Table I for the hydrolysis of 900 grams of sucrose per liter at 100°C. by citric acid in the concentration stated is given below. Samples of the calculations of k and the per cent of sucrose remaining are given below.

TABLE I. SHOWING HYDROLYSIS AT 100°C. OF 90 g. SUCROSE BY 1 c.c. 7.074 N CITRIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% sucrose remaining	Log of % sucrose remaining	k	k'
0	0	67.3	100	2.0000	-----	-----
1	2	42.5	71.9	1.8567	0.1650	0.1650
2	4	23.3	50.1	1.6998	0.1728	0.1806
3	6	8.05	32.8	1.5159	0.1858	0.2117
4	8	-2.1	21.3	1.3284	0.1933	0.2159
5	10	-8.1	14.5	1.1614	0.1931	0.1923
6	13	-14.3	7.48	0.8739	0.1995	0.2207
7	16	-17.9	3.40	0.5315	0.2113	0.2628
8	19	-19.0	2.16	0.3345	0.2018	0.1512
9	22	-19.98	1.04	0.0170	0.2075	0.1828
10	26	-20.9	0	-----	-----	-----
11	30	-20.9	0	-----	-----	-----
					Ave. k = 0.1922	Ave. k' = 0.1981

Sp. Gr. at 20°C./20°C. = 1.3343.

Calculations of the per cent sucrose remaining and k are given for the 8 minute interval.

$$\% \text{ sucrose} = \frac{-2.1 - (-20.9)}{67.3 - (-20.9)} \times 100 = \frac{18.8}{88.2} \times 100 = 21.3\%.$$

$$k = \frac{2.3026}{8} (2.0000 - \log 21.3) = \frac{2.3026}{8} (2.0000 - 1.3284) = 0.1933.$$

Values of k in parenthesis in any of the following tables were not used in the evaluation of the average k .

TABLE II. SHOWING HYDROLYSIS AT 100° OF 80 g. SUCROSE BY 1 c.c. 7.074N CITRIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % Sucrose remaining	k
0	0	59.55	100.0	2.0000
1	2	38.5	72.4	1.8597	0.1615
2	4	24.7	54.3	1.7348	0.1527
3	6	13.36	39.4	1.5955	0.1552
4	8	2.8	25.6	1.4082	0.1703
5	10	-3.97	16.7	1.2227	0.1790
6	13	-10.0	8.79	0.9440	0.1870
7	16	-13.1	4.72	0.6739	0.1908
8	20	-14.9	2.36	0.3729	0.1873
9	25	-16.56	0.18	1.2553	0.2528
10	30	-16.7	0.0
					Ave. k = .1818

Sp. Gr. 20°C./20°C. = 1.2982.

TABLE III. SHOWING HYDROLYSIS AT 100° C. OF 70 g. SUCROSE BY 1 c.c. 7.074N CITRIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % Sucrose remaining	k
0	0	53.6	100.0	2.0000
1	5	21.1	52.2	1.7177	(0.1300)
2	10	-1.3	19.4	1.2878	0.1678
3	15	-9.3	7.57	0.8791	0.1721
4	20	-12.9	2.28	0.3579	0.1890
5	25	-13.65	1.17	0.0682	0.1780
6	30	-14.1	0.51	1.7076	0.1760
7	35	-14.4	0.07	2.8451	0.2076
8	40	-14.45	0
9	45	-14.45
					Ave. k = .1817

Sp. Gr. 20°C./20°C. = 1.2653.

TABLE IV. SHOWING HYDROLYSIS AT 100°C. OF 60 g. SUCROSE BY 1 c.c. 7.074N CITRIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	44.1	100.0	2.0000	-----
1	2	27.4	71.0	1.8513	0.1712
2	4	13.7	47.4	1.6758	0.1866
3	6	3.9	30.4	1.4829	0.1984
4	8	-2.25	19.8	1.2967	0.2024
5	10	-6.6	12.3	1.0899	0.2096
6	13	-10.0	6.4	0.8062	0.2115
7	16	-12.0	2.94	0.4683	0.2204
8	19	-12.9	1.38	0.1399	0.2254
9	22	-13.15	0.95	1.9777	0.2117
10	26	-13.6	0.17	1.2305	0.2452
11	30	-13.7	0.0	-----	-----
					Ave. k = 0.2082

Sp. Gr. at 20°C./20°C. = 1.2264

TABLE V. SHOWING HYDROLYSIS AT 100°C. OF 40 g. SUCROSE IN 1 c.c. 7.074N CITRIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	30.2	100.0	2.0000	-----
1	5	6.4	39.3	1.5944	0.1872
2	10	4.0	12.77	1.1062	0.2058
3	15	-7.2	4.60	0.6628	0.2053
4	20	-8.5	1.28	0.1072	0.2179
5	25	-8.6	1.02	0.0086	0.1834
6	30	-8.95	0.13	1.1139	0.2215
7	35	-8.95	0.13	1.1139	0.1899
8	40	-8.95	0.13	1.1139	0.1661
9	45	-9.00	0.0	-----	-----
					Ave. k = 0.1971

TABLE VI. SHOWING HYDROLYSIS AT 100°C. OF 80 g. SUCROSE BY 2 c.c. 7.074 N CITRIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% sucrose remaining	Log of % sucrose remaining	k
0	0	58.8	100.0	2.0000	-----
1	2	26.7	58.3	1.7657	0.2698
2	4	4.7	29.7	1.4728	0.3035
3	6	-6.9	14.6	1.1644	0.3207
4	8	-12.9	6.77	0.8306	0.3366
5	10	-15.6	3.25	0.5119	0.3426
6	13	-17.6	0.65	1.8129	0.3077
7	16	-18.1	0.0	-----	-----
8	20	-18.1	-----	-----	-----
					Ave. k = 0.3135

Sp. Gr. at 20°C./20°C. = 1.3022.

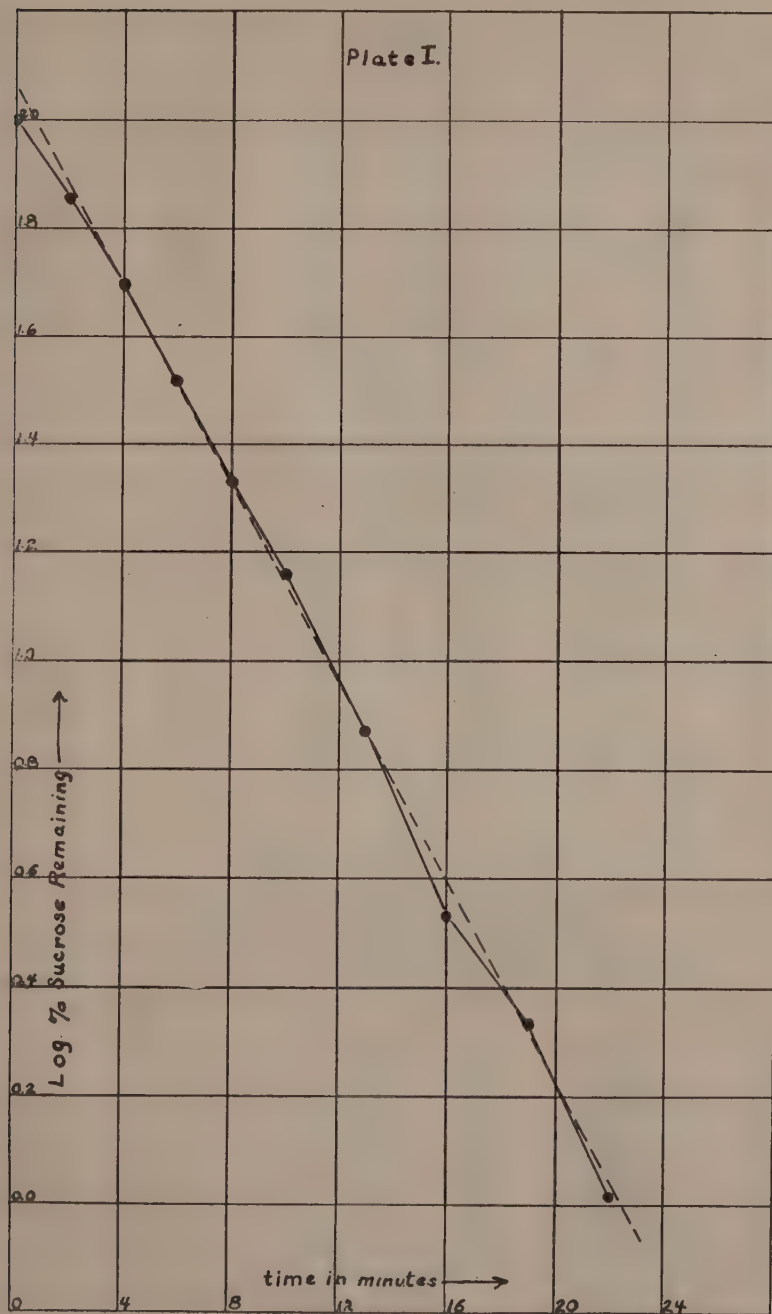


TABLE VII. SHOWING HYDROLYSIS AT 100°C. OF 80 g. SUCROSE BY 1 c.c. 6.553N TARTARIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % Sucrose remaining	k
0	0	60.3	100.0	2.0000
1	2	35.0	67.2	1.8274	0.2068
2	4	13.1	38.7	1.5877	0.2373
3	6	0.9	22.8	1.3579	0.2464
4	8	-7.1	12.47	1.0959	0.2602
5	10	-11.3	7.02	0.8463	0.2657
6	13	-14.5	2.86	0.4564	0.2734
7	16	-15.9	1.04	0.0170	0.2854
8	20	-16.7	0.0
9	25	-16.7	0.0
					Ave. k = 0.2536

Sp. Gr. at 20°C./20°C. = 1.2992.

TABLE VIII. SHOWING HYDROLYSIS AT 100°C. OF 8 g. SUCROSE BY 2 c.c. 6.553N TARTARIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	59.8	100.0	2.0000
1	2	20.4	48.8	1.6884	0.3588
2	4	-2.3	19.5	1.2900	0.4087
3	6	-11.7	7.27	0.8615	0.4369
4	8	-15.4	2.47	0.3927	0.4626
5	10	-16.5	1.04	0.0170	0.4566
6	13	-17.3	0.0
7	16	-17.3	0.0
					Ave. k = 0.4247

Sp. Gr. at 20°C./20°C. = 1.3005

TABLE IX. SHOWING HYDROLYSIS AT 30°C. OF 80 g. SUCROSE BY 1 c.c. 7.074N CITRIC ACID IN 100 c.c. SIRUP.

Date	Time	Time of hydrolysis in days	Saccharimeter readings	% sucrose remaining	Log of % sucrose remaining	k ($\times 10^{-5}$)
Feb. 3, 1927	9:19 A. M.	0	60.3	100.0	2.0000
Feb. 5	9:54 A. M.	2.03	46.1	81.4	1.9106	7.05
Feb. 7	3:31 P. M.	4.25	36.0	68.3	1.8344	6.23
Feb. 10	10:23 A. M.	7.04	23.25	51.6	1.7126	6.49
Feb. 13	4:31 P. M.	10.30	13.0	38.2	1.5821	6.49
Feb. 17	2:12 P. M.	14.21	2.7	29.7	1.4728	5.94
Feb. 25	2:50 P. M.	22.23	-6.2	13.1	1.1173	6.35
Mar. 4	1:44 P. M.	29.19	-12.4	4.97	0.6964	7.15
Mar. 17	8:30 P. M.	41.97	-16.1	0.13	1.1139
Mar. 26	3:13 P. M.	51.25	-16.2	0.0
						Ave. k = 6.53 $\times 10^{-5}$

Sp. Gr. at 20°C./20° = 1.3023.

TABLE X. SHOWING HYDROLYSIS AT 30°C. OF 80 g. SUCROSE BY 2 c.c. 7.074N CITRIC ACID IN 100 c.c. SIRUP.

Date	Time	Time of hydrolysis in days	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k ($\times 10^{-3}$)
Feb. 3, 1927	9:26 A. M.	0	60.3	100.0	2.000
Feb. 5	9:56 A. M.	2.03	41.4	76.1	1.8814	(9.35)
Feb. 7	3:32 P. M.	4.25	28.0	59.1	1.7716	8.59
Feb. 10	10:25 A. M.	7.04	13.1	40.3	1.6053	8.91
Feb. 13	4:33 P. M.	10.3	3.6	23.2	1.4502	6.54
Feb. 17	2:15 P. M.	14.2	-5.2	17.1	1.2330	8.64
Feb. 25	2:52 P. M.	22.23	-13.3	6.84	0.8351	8.40
Mar. 4	1:46 P. M.	29.19	-16.5	2.79	0.4456	8.52
Mar. 17	8:32 A. M.	41.97	-18.7	0.0	Ave. k =
Mar. 26	3:15 P. M.	51.25	-18.7			8.60×10^{-3}

Sp. Gr. at 20°C./20°C. = 1.3023.

TABLE XI. SHOWING HYDROLYSIS AT 90° OF 80 g. SUCROSE BY 1 c.c. 7.074N CITRIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	59.8	100.0	2.0000
1	5	37.7	71.5	1.8543	0.0671
2	10	20.4	49.2	1.6920	0.0709
3	15	7.8	33.0	1.5185	0.0738
4	20	-0.3	22.75	1.3570	0.0740
5	25	-6.4	14.7	1.1673	0.0767
6	30	-10.3	9.66	0.9850	0.0779
7	35	-12.9	6.32	0.8007	0.0789
8	40	-14.9	3.74	0.5729	0.0821
9	50	-16.9	1.16	0.0645	0.0891
10	60	-17.7	0.13	1.1139	(0.1109)
11	70	-17.8	0.0
					Ave. k = 0.0767

Sp. Gr. at 20°C./20°C. = 1.3012.

TABLE XII. SHOWING HYDROLYSIS AT 90°C. OF 80 g. SUCROSE BY 1 c.c. 6.553N TARTARIC ACID IN 100 c.c. SIRUP.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% sucrose remaining	Log of % sucrose remaining	k
0	0	59.9	100.0	2.0000
1	5	31.3	63.6	1.8035	0.0905
2	10	11.3	38.1	1.5809	0.0965
3	15	-1.2	22.2	1.3463	0.1004
4	20	-8.5	12.85	1.1089	0.1026
5	25	-12.9	7.3	0.8633	0.1047
6	30	-15.7	3.7	0.5682	0.1099
7	35	-16.8	2.3	0.3617	0.1078
8	40	-17.6	1.28	0.1072	0.1090
9	50	-18.6	0.0
10	60	-18.6	0.0
11	70	-18.6	0.0
					Ave. k = 0.1027

Sp. Gr. at 20°C./20°C. = 1.3007.

A comparison of the values of k in any of the tables will show that, in general, k increases as the reaction progresses. This is in agreement with the results of Pennycook²⁰, but it disagrees with the conclusions of Spencer.¹² However, there seems to be little uniformity in the increase in value. Plate I shows the curve obtained by plotting the logarithm of the per cent of sucrose remaining against time from data in Table I. The broken line is the best straight line which can be drawn through these points. From the position of the points with reference to this line it appears that the reaction approaches that of a monomolecular one as a limit. The slope of the broken line is equal to 0.2114, which is the value of k for the ideal monomolecular reaction corresponding to these data.

If the value of k is calculated from point to point of the curve instead of from the initial concentration of sucrose each time, there seems to be more fluctuation in k . Values of k figured in this way are given in the column headed k' in Table I. k' , since it is calculated from point to point of the curve, is subject to wider fluctuation than the k calculated by the usual method, since small experimental errors will cause a greater change from the average. If the k values are always based on the initial concentration, the fluctuations will not be as great.

A probable reason for the increasing value of k as the reaction progresses is to be found in the water content. In the sirup considered in Table I, there are 900 grams of sucrose as compared to about 434 grams of water. In terms of mols there are 2.63 mols of sucrose and 24.0 mols of water originally. After hydrolysis has occurred, since each molecule of sucrose causes the disappearance of one molecule of water, there will be a

$$\frac{2.63}{24.0} \times 100 = 10.9\% \text{ decrease in the concentration of the water present.}$$

This reduction in solvent should partially explain, at least, the tendency for the rate of reaction to increase as the hydrolysis progresses.

The concentration of sucrose, in the experiments described in Tables I to V, was varied from 900 grams to 400 grams per liter. Increase in the concentration of sucrose from 400 to 600 grams per liter causes an increase in the value of the average k . For concentrations of sucrose between 600 and 900 grams per liter, there is no regularity to the effect on the rate of reaction caused by an increase in the concentration of sucrose. No general statement can be made concerning the effect of increasing the concentration of sucrose upon the rate of reaction.

Osaka²³ has shown that the inversion of sucrose by acids is incomplete in high concentrations although it may be regarded as complete in dilute solutions. For a sirup consisting of 800 grams of sucrose per liter of sirup, he says inversion is 98.9% complete when using a mineral acid for inversion at 25°C. Osaka considers that it is very probable that in a concentrated solution a reversion of sucrose from its hydrolytic products takes place.

Determination of the amounts of invert sugar in equilibrium mixtures by the Munson-Walker gravimetric Fehling's method²⁴ before and after inversion by the Tucker²⁵ method showed inversion to be about 97.5% complete for sirups originally containing 800 grams of sucrose per liter. It is

not surprising that the equilibrium point is not the same as that reported by Osaka since he used mineral acids in his inversion process while citric and tartaric acids were used in these studies.

Sale and Skinner⁴ say that complete inversion was not obtained in a 30° Baumé sirup when tartaric acid was used. They report an inversion of 96% when 0.021% by weight of tartaric acid crystals (99.5%) based on the weight of sugar and water taken, is added to a 30° Baumé sirup which was subsequently boiled for 40 minutes. The addition of 0.013% of hydrochloric acid followed by boiling for 20 minutes was sufficient to cause practically complete inversion.

Jordan²⁶ gives directions for the preparation of commercial invert sugar sirups. On a 1000 pound producing basis, he advises the use of 750 pounds of sucrose, 250 pounds of water (about 30 gallons), and 10 ounces of tartaric or 13 ounces of citric acid crystals. The whole mixture is heated at incipient boiling for 30 minutes. Jordan claims 98% completeness of inversion for this process.

Ruche²⁷ advises the use of 50 grams (about 1.76 ounces) of tartaric acid crystals with 100 pounds of sucrose and 45 pounds of water. This should be boiled for 30 to 35 minutes. He makes the statement that invert sugar is sweeter than the sucrose from which it is prepared and advises the preparation of invert sugar as an economy measure for ice cream manufacturers. References have previously been cited to show that he was in error in his statement regarding the sweetness of invert sugar.

In the preparation of invert sugar sirups the time of boiling may be reduced by increasing the concentration of acid. If large quantities of the invert sugar sirup are to be prepared, steam-jacketed kettles are generally used. Since it takes ten to fifteen minutes of heating in the average steam kettle to bring its sirup contents to the boiling temperature, much inversion would be accomplished in that time. Then by boiling for 5 minutes, the inversion would be completed or equilibrium attained. The boiling points of concentrated sirups may be as high as 115°C. Calculations made with the aid of the van't Hoff isochore using data from Tables VII and XII showed 5.5 minutes as the time for 99% completeness of inversion at 110°C.

In a previous paper, number I of this series, data have been given which show that sterilization of sirups to which acid has been added will have been effected in less than 5 minutes at the boiling temperature. Obviously, if the production of an invert sugar sirup is desired, it will also be sterilized simultaneously.

SUMMARY

1. Simultaneous inversion and sterilization of sucrose sirups may be advantageously accomplished by the addition of one cubic centimeter of 7.074 N citric or 6.553 N tartaric acids per 100 cubic centimeter quantities of sirup followed by heating the sirup to the boiling point and continuing the boiling process for 5 minutes.

2. Complete inversion is not reached when citric acid or tartaric acids are used to invert concentrated sucrose sirups. An equilibrium occurs when about 97.5% of complete inversion has taken place in concentrated sirups.

3. The monomolecular reaction k is not a constant for the hydrolysis of sucrose sirups. In general, k increases as the hydrolysis progresses.

4. No general statement can be made concerning the effect of increasing the concentration of sucrose upon the rate of reaction for concentrations of sucrose greater than 600 grams per liter. The rate of inversion increases slightly with increasing concentration of sucrose up to 600 grams per liter.

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STUDIES IN THE BACTERIOLOGY OF SULPHUR STINKER SPOILAGE OF CANNED SWEET CORN

C. H. WERKMAN and HELEN J. WEAVER.

From the Bacteriology Section, Iowa Agricultural Experiment Station, Ames.

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The canning industry of the middle western states experiences sporadic and costly losses from a blackening of canned sweet corn known in the trade as "sulphur stinker" spoilage. The condition is characterized by the progressive development of a marked blackening of the kernels, particularly of the germs, and the evolution of a nauseating odor of hydrogen sulphid gas. It is distinct from spoilage known as "metallic" or "chemical" blackening, a condition affecting principally the heads and seams of the cans, and is readily differentiated from the latter by the appearance and uniform distribution of the blackened germs throughout the contents. The sulphur stinker condition is readily recognized and is not easily confused with other types of spoilage.

Outbreaks of the trouble occur sporadically in a cannery and generally involve a substantial part of the season's pack; in several instances the entire pack for the season has been so heavily contaminated that salvage was impracticable. The evidence is also strongly in favor of the view that the cause of sulphur stinker spoilage in sweet corn is identical with that of a similar spoilage occurring in canned peas. At least, the microorganism which we have shown to be responsible for sulphur stinker spoilage in sweet corn attacks canned peas, producing a condition very similar to that occurring in sweet corn.

The incidence of spoilage among the cans of a contaminated pack generally runs high. No accurate information covering a large number of outbreaks is available, although the losses involved in several outbreaks occurring in Iowa have been heavy. In 1919 an Iowa cannery lost practically its entire pack of sweet corn for the season, while in 1925 another Iowa cannery experienced a very heavy infection in a two days' pack. The present studies were carried out on a pack in which the incidence of spoilage among the cans was estimated at 25 percent, while the 150 cans examined from this pack, although known to be heavily infected, gave an incidence of 55 percent.

An individual spoiled can is referred to as a "sulphur stinker". The appearance of such a can is normal; there is no swelling, bulging or springing. In fact, the can loses little or none of its vacuum. The sulphur stinker judged by its appearance cannot be differentiated from the normal can of sweet corn. Detection of the spoilage depends, therefore, upon an examination of the contents.

Upon opening the sulphur stinker, one notices an odor of hydrogen sulphid and observes floating in the blue-grey liquid numerous separated germs blackened by the action of the sulphid on the iron salts in the germ. That the blackening is due to a sulphid of iron would seem to be demonstrated by the potassium ferrocyanide and potassium thiocyanate tests for iron which indicate the presence of iron salts in the corn germ. The odor

given off by the sulphur stinker is that only of hydrogen sulphid gas, and the presence of a putrefactive odor is not discernable.

It is of interest to note that the corn examined was prepared "Maine style" or "cream style" as it is sometimes known. This method of preparation, in which the scrapings from the cob are added to make a product of creamy consistency, permits of the presence of numerous broken germs, a condition we have found conducive to the development of the blackening. "Maryland style" corn, in which only the whole kernels are used, is much more resistant to the development of the blackening.

Certain difficulties in the detection of sulphur stinker spoilage and the inability of the canner to determine the presence of the organism causing the trouble increase the losses. The development of the individual spoiled cans is irregular; under ordinary temperatures occurring after processing some cans develop the sulphur stinker condition over-night, while others require as long as two or three weeks. This with the fact that there is no external evidence of spoilage increases the difficulties of detection and salvage. At the present time there is no satisfactory or practicable method of salvaging the unspoiled cans in a contaminated pack. Puncturing the cans near the head and determining the effect of the expelled gas upon moist lead acetate paper has been tried, but the method has been found to be expensive and time consuming.

With the cause of sulphur stinker spoilage established, it is possible that satisfactory methods of detecting the presence of the organism responsible may be developed, which will serve to indicate foci of infection in the plant. Such methods would be particularly applicable should it prove impracticable to control the development of the organism by adequate cooling and maintenance of the corn in storage at sufficiently low temperatures.

The present investigation constitutes a study of the bacteriology of sulphur stinker spoilage. It involves the isolation, classification and tentative naming of the organism responsible for the spoilage; the physiological and cultural characteristics of the organism and possible methods of control based upon these characteristics are considered in some detail. The habitat of the organism and its bearing on the ability of the organism to gain access to the corn are considered, as is the effect of the organism on a number of canned vegetables.

We have found no record of studies on sulphur stinker spoilage in the literature.

MATERIAL AND METHOD

The experimental part of this investigation is based upon a study of twelve cases of twenty-four cans each of sweet corn of an Iowa cannery taken from a pack which was known to be heavily infected during the 1926 season. The incidence of spoilage among the 12 cases as determined by an examination of 150 cans taken at random was 55 percent. The corn used by this company was Country Gentleman, purchased from farmers in the vicinity of the cannery and prepared "Maine style" of medium consistency.

The method used in preparing the canned corn is as follows: The corn is husked upon a wood slat conveyor, the ears then pass on this conveyor, are transferred to a steel conveyor and in turn are again transferred to a steel bucket elevator in which they are elevated to the third floor of the cannery. This bucket elevator discharges into a galvanized iron chute,

through which the ears fall upon a rubber belt. This rubber belt conveys the ears past the sorters, who sort out the defective and mature ears and make a separation of the ears for the purpose of grading the corn into fancy and standard grades. The standard corn is then diverted into a wooden crib. This crib is kept well protected by hard, white paint. The cut corn is discharged into a galvanized screw conveyor, which conveys the corn to the mixing tank. This mixing tank is made of copper and heavily tinned. From the upper mixing tank the corn falls to a lower tank, which is of similar construction. From this it is passed into a regular Sprague-Sells Filler.

All the equipment used at this plant, with the exception of the slat conveyor in the husking shed, was installed new for the 1926 season. All of it may be termed modern sanitary cannery equipment.

The cans are filled at a temperature which insures an initial temperature of 160°Fahr. (71°C.), followed by processing at 245°Fahr. (118°C.) for 70 minutes.

The cooling system employed is known as the "slat conveyor cooling tank" method. The cans to be cooled are dumped from crates in which the cans were processed and passed through the canal on the conveyor running at the bottom of the tank.

The storage temperature will vary from 90° to 130° F. (32° to 54° C.) in the individual cases during the first week to ten days after packing. The corn then gradually cools to the prevailing atmospheric temperature during the fall months. In the winter months the storage rooms are heated in order to prevent freezing of the corn.

Several of the experimental cans were incubated at 37° C. and at 55° C. for long periods, but no changes were observed.

Each can was carefully examined for defects before opening, washed and the top flamed while the can was held inverted. The can opener was likewise sterilized.

When the spoiled cans were opened there was a strong odor of hydrogen sulphid. The contents varied in color from a bluish-grey to black, depending upon the extent of spoilage. Loose blackened corn germs floated on the surface of the grey liquid. The inside tin of the cans was blackened in the more severe cases. Determinations made on 15 spoiled and 5 unspoiled cans gave a pH of 6.3 to 6.4 for both sets.

Details of the experimental methods employed will be given under experimental results.

EXPERIMENTAL

The characteristics of sulphur stinker spoilage suggested that micro-organisms were responsible for the trouble. It was first determined that the condition could be transmitted from can to can by inoculating five cubic centimeters of spoiled corn into unspoiled corn and incubating at 55° C. The condition did not occur when the temperature of incubation was 30° C. and developed slowly at 37° C. Even with an incubating temperature of 55° C. the development of sulphur stinker spoilage was exceedingly irregular. Growth appeared to be better in corn which had been heated for several minutes at 100° C. before being inoculated. Some of the inoculated tubes developed a typical sulphur stinker condition within 24 hours, whereas others developed it only after two or three weeks. Some of the tubes in

variably failed to show any signs of spoilage even after a month's incubation. However, the typical spoilage was transmitted through numerous transfers, which strengthened the belief in the biological cause of the trouble.

Microscopic examination of the spoiled corn from the original cans revealed very few organisms. Occasionally a gram positive rod and what appeared to be spores were observed scattered widely over the slide preparations. The gram positive rods and the oval spores were the only forms found in the spoiled cans. Microscopic examination of young cultures inoculated from the original cans revealed gram positive rods from 3 to 6 micra long and 0.5 micron in diameter with rounded ends and subterminal oval spores which hardly swelled the vegetative cells. Young cultures revealed the organism as motile. The motility suggested peritrichous flagellation. Flagella were not demonstrated by staining methods. Motility was difficult to demonstrate due apparently to the fact that it occurs in young cultures and during certain stages of growth only. The vegetative cells themselves showed a marked ability to stain granularly. From one to six gram positive spherical granules were often observed in 24 hour cultures which had been incubated at 55° C. Young spores retained the gram stain; older spores failed to take the gram stain.

Several cultures of the organism were isolated from the original cans of spoiled corn by anaerobic methods. These were repeatedly purified by cultural methods.

Considerable difficulty has been experienced in preparing a medium which would consistently furnish a luxuriant growth of the organism. Numerous formulae for media suitable to the growth of anaerobes were tried, but none was found to produce a growth markedly superior to that of the rest. Beef heart infusion agar adjusted to pH 7.2 was finally chosen as one of the stock media. To the basic media was generally added 0.1 percent ferric chloride to produce a more marked blackening of the medium. The organism has remained fastidious in its choice of a medium and one is never quite certain that a transfer will develop. It has shown an aversion toward growing on the surface of media and it was only after the inoculated medium had been covered by a heavy layer of petrolatum and incubated in an atmosphere of hydrogen that isolated surface colonies were obtained. It has seemed to us that oxygen relationships played a very important part in the success of the culture. Beef heart infusion agar plates inoculated heavily and incubated at 55° C. in an atmosphere of hydrogen have failed to produce growth; the pyrogallie acid and sodium hydroxide method was likewise unsuccessful. On the other hand, shake cultures in beef heart infusion gave good colony growth as indicated by the blackening of the medium around the colony, although microscopic examination of these colonies revealed relatively few organisms present. Growth in capillary tubes filled with beef heart infusion agar was successful in isolating colonies of the organism. In all cultural studies boiling the medium for a period of time before inoculation gave better results. This characteristic of the organism was first noticed in thermal death determinations. It was repeatedly observed that tubes of medium which had been heated for long periods of time showed the most rapid growth.

Although some dozen cultures of the organism from different cans were isolated, accurate information has been obtained on only four cultures.

All grew on a variety of media under suitable conditions. These conditions were not easily determined and not consistently duplicated. A medium of finely ground peas (200 grams), dibasic potassium phosphate (1 gram), ferric chloride (trace), water (1 liter), and adjusted to pH 7.2 proved to be a good medium and was used for one set of cultures.

PHYSIOLOGICAL CHARACTERISTICS OF THE ORGANISM RESPONSIBLE FOR SULPHUR STINKER SPOILAGE

Action on carbohydrates, glucosides and alcohols. The sulphur stinker organism did not attack any of 28 sugars, alcohols or glucosides with the production of acid or acid and gas. The following substances were used in beef heart infusion and the tests repeated in the pea medium: glucose, sucrose, lactose, raffinose, arabinose, xylose, levulose, maltose, galactose, mannose, rhamnose, trehalose, melezitose, adonitol, inositol, dulcitol, erythritol, perseitol, arabitol, sorbitol, glycerol, inulin, dextrin, salicin, amygdalin, esculin, glycogen and starch. The organism is strictly non-saccharolytic.

Action on gelatin and serum. The organism is only feebly proteolytic. Gelatin was not liquefied nor was serum digested. Cystine was attacked with the production of hydrogen sulphid. Gelatinolysis was determined by two methods. Frazier's (1926) method was modified as follows in order to use it for this organism: beef heart infusion agar was prepared containing the infusion from 50 grams of beef heart per liter, 0.4 percent gelatin and a trace of ferric chloride. The medium was inoculated at 45° C. and drawn into capillary tubes to harden. It was then incubated at 55° C. until the colonies had developed. Long periods of incubation were required with this medium. The core of agar, containing colonies, was then blown into an acid solution of bichloride of mercury (1:500). The presence of a clear zone around a colony would indicate decomposition of the gelatin at least as far as the peptone stage. The width of the zone would be indicative of the amount of liquefaction. Essentially the same procedure was repeated, substituting 1 percent tannic acid for the bichloride solution.

Gelatinolysis was also determined by incubating cultures of the organism at 55° C. in beef heart infusion agar containing 12 percent gelatin. Cultures were removed at intervals and cooled to determine liquefaction, but none was observed during one month's incubation.

Upon Loeffler's blood serum, inoculated, covered with petrolatum and incubated at 55° C., colonies developed, but there was no observable digestion of the medium.

Upon von Hibler's (1924) brain medium the colonies were small, round and black.

Indol was not produced as determined by growth in pea medium or beef heart infusion using the paradimethylamidobenzaldehyde test to indicate indol.

Reduction of nitrates. Nitrates were not reduced to nitrites. Beef heart infusion and pea medium with 0.1 percent potassium nitrate were inoculated, incubated at 55° C. for one, two and three days and tested for the presence of nitrites.

Growth temperature relationships. The sulphur stinker organism is a true thermophile growing at an optimum temperature of 55° C., a maximum of 65° or 70° C. and a minimum of 30° C. Tubes containing 30 c.c. of beef heart infusion agar were inoculated with 5 c.c. each of a culture of the organism and six tubes were incubated at each of various temperatures: Growth at 50° and 55° C. occurred over-night; at 37° and at 45° C. growth occurred during 48 hours; at 60° and 65° C. growth was meager, while at 70° C. there was rarely development.

Thermal death times. The time required to kill the spores of the organism at a definite temperature is of particular importance to the canning industry.

Our results consistently showed that eight hours' exposure at a temperature of 100° C. was required to destroy the spores of the organism at pH 7.0. Heated at 118° C. the organism survived 50 minutes at pH 7.0 and 40 minutes at pH 6.3. In processing at 118° C. for 70 minutes, the interior of the can reaches a maximum temperature of approximately 115° C. (239° Fahr.). Fig. 3 was kindly furnished us by Mr. W. H. Harrison, Director of the Research Dept. of the Continental Can Company. It is apparent that the processing could not be expected to destroy the sulphur stinker organism. Many canneries process at 121° C. (250° Fahr.). At this temperature the organism survived 35 minutes heating at pH 7.0 and 25 minutes at pH 6.3. The processing temperature could not be raised in order to destroy the organism since it would have to be raised considerably and this would lead to undesirable flavors in the product.

The thermal death times were determined as an index of the resistance of the organisms to heat effects. The time required to kill equal numbers of organisms is a measure of the resistance of the organism to heat effects. The thermal death times were determined in the following manner: One cubic centimeter of a centrifuged pea medium culture of the organism adjusted to the desired pH and diluted so as to contain approximately 8,000,000 spores, was sealed in a thin glass test tube. Centrifuging was regulated so as to throw down particles of pea material in the medium, but not the organisms. The sealed ampoules containing 1 c.c. of the culture were brought to a temperature of 65° C. in order to reduce the time required to bring them up to the killing temperatures. They were then placed in the salt water or oil bath at the temperature desired. A motor stirrer circulated the brine or oil. Duplicate tubes were removed after definite intervals, plunged into cold water, then inoculated into pea medium or beef heart infusion medium and incubated at 55° C. Table 1 gives the results of an experiment.

pH range of growth. The optimum growth of the organism occurred at pH 7.2, with the minimum at pH 5.8 and the maximum at pH 7.6.

Beef heart infusion agar adjusted at intervals of 0.2 pH value from pH 5.0 to pH 8.0 was heavily inoculated at 45° C. and drawn into capillary tubes, allowed to harden and incubated at 56° C. Below pH 5.8 and above pH 7.6 the organism failed to grow. The optimum lay near pH 7.2.

Pathogenicity. The organism was not pathogenic when ingested by man, guinea pig, mouse, rat or rabbit. Introduced parenterally into the guinea pig, mouse, rat or rabbit the organism produced no general symptoms or cutaneous lesions. As much as 5 c.c. of pea medium cultures were

introduced intraperitoneally into rabbits, guinea pigs and rats; mice received one cubic centimeter injections.

Production of agglutinins. In a study of anaerobes Le Claine and Morel (1901) found that the injection of bacilli into animals generally led to the production of agglutinins. Since then the agglutination reaction has been found of considerable value in the differentiation of anaerobes. *Clostridium welchii* and *Clostridium tertium* are apparently exceptions in that the use of their sera has not given agglutination reactions.

Preparation of a satisfactory antigen for the macroscopic agglutination when injected into rabbits. The animals were injected intraperitoneally with one cubic centimeter of pea medium culture every fourth day until four such injections had been made. The animals were bled 10 days after the last injection.

Preparation of a satisfactory antigen for the microscopic agglutination test was impracticable because of the few organisms present in the culture. The microscopic test was therefore resorted to. Agglutination was observed in a 1 to 500 serum dilution. Specificity tests were not attempted at this time.

Classification and nomenclature of Clostridium nigrificans. Cultural and morphological characteristics of the organism responsible for sulphur stinker spoilage place it in the genus *Clostridium*. Bergey (1925) characterizes the genus as "anaerobes or microaerophiles, often parasitic rods, commonly enlarged at sporulation and producing clostridial or pleotridial forms."

Search of the literature has failed to reveal a description of an organism having the characteristics of the one responsible for sulphur stinker spoilage. Von Hibler (1908) described a group of sporulating anaerobes with oval terminal spores. This group is probably the nearest approach to the sulphur stinker organism. He designated these forms as group IX, but made no exhaustive study of them. McIntosh (1917-1918) described several strains which conformed in general to Von Hibler's group IX. His type III-C most closely resembles the sulphur stinker organism. Douglass, Fleming and Colebrook (1920) in an unpublished report to the Medical

TABLE 1. THERMAL DEATH TIMES AT VARIOUS TEMPERATURES.

Time in Minutes	100° C.	118° C.		121° C.	
	pH 7.0	pH 7.0	pH 6.3	pH 7.0	pH 6.3
15	+	+	+	+	+
20	+	+	+	+	+
25	+	+	+	+	+
30	+	+	+	+	—
35	+	+	+	+	—
40	+	+	+	—	—
45	+	+	—	—	—
50	+	+	—	—	—
55	+	—	—	—	—
60	+	—	—	—	—
65	+	—	—	—	—
70	+	—	—	—	—
420	+				
435	+				
450	+				
465					
480					

TABLE 2. EFFECT OF *CL. NIGRIFICANS* ON VARIOUS CANNED VEGETABLES.

Vegetable	pH	Blackening by <i>Cl. nigrificans</i>			
		Original cans	0.1% FeCl ₃	Reaction adjusted to pH 7.0	
Asparagus	5.7	—	—	—	—
Beans					
Wax	6.3	—	B	—	B
Green	6.4	—	B	—	B
Bean Hole	6.8	B	B	B	B
Beets	5.4	—	—	B	B
Carrots	5.2	—	—	B	B
Corn (Sweet)	6.3	B	B	B	B
Hominy	6.8	B	B	B	B
Lima Beans	5.8	—	—	—	—
Peas	6.3	B	B	B	B
Pumpkin	5.2	—	—	B	B
Spinach	5.2	—	—	—	—
Sweet Potato	5.2	—	—	—	—

Research Council, gave the name *Bacillus cochlearius* to McIntosh's type III-C organism.

According to McIntosh, *Bacillus cochlearius* is a sporulating bacillus frequently found in war wounds. It is an actively motile, slender rod, variable in length and having a tendency to give up the gram stain. The spores are strictly terminal and oval when fully developed, giving the organism a spoon-shape appearance.

From the description of *Bacillus cochlearius* available it appears that it is a mesophile. Furthermore, the inability of *B. cochlearius* to produce hydrogen sulphid or blackening of brain media serves to differentiate *B. cochlearius* from the sulphur stinker organism. Neither organism has

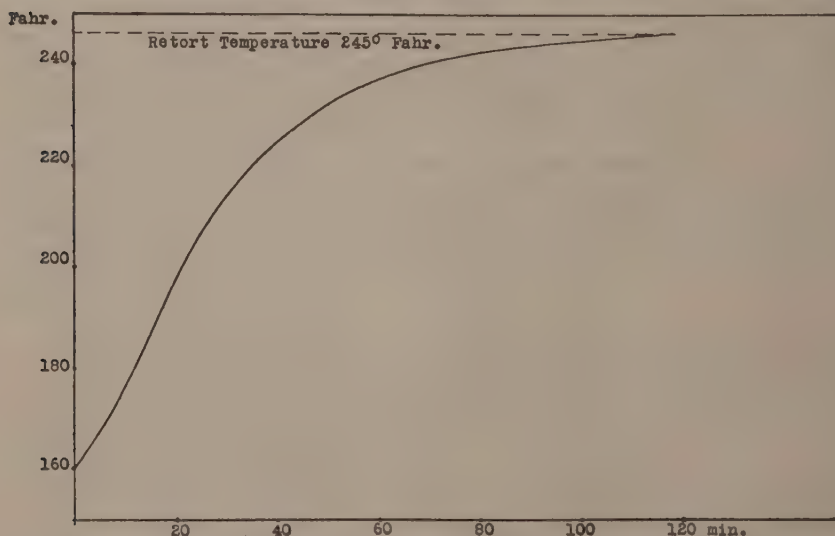


Fig. 3. Heat penetration curve of No. 2 can of corn.

saccharolytic properties and neither liquefies gelatin or coagulates blood serum.

Considerable confusion exists in the literature regarding the classification of the anaerobes and it is not the intention to add to it. On the other hand, it would seem that the differences pointed out are of sufficiently basic character to warrant giving specific rank to the organism. The name *Clostridium nigrificans* is proposed.

Ability of Clostridium nigrificans to produce spoilage in various canned vegetables. It would seem of value to know the effect of *Clostridium nigrificans* when introduced in pure culture form into various canned vegetables.

The ability of the organism to produce a condition in canned peas similar to sulphur stinker spoilage in canned corn has been noted. Peas are uniformly blackened if the seed covering is broken; if the covering is not broken, blackening is slow to develop. Relatively few crushed peas in a can will allow darkening of the contents. The blackening of peas is more marked than in the case of sweet corn. Ground peas afford a favorable medium for the growth of the organism. In Table 2 are given the results of inoculating pure cultures of *Clostridium nigrificans* into various canned vegetables as they are purchased in the market, and with the pH adjusted to 7.0. The effect of adding an iron salt is also shown. From these results it would seem that canned peas and sweet corn offer the only opportunities for the development of spoilage. The acidity is the controlling factor in the case of many canned vegetables. Hominy and Bean Hole Beans produce a colony growth in which a few black colonies develop with no tendency to spread or cause a general blackening appearance. The methods of preparing these two vegetables would probably preclude sulphur stinker spoilage.

Habitat of Clostridium nigrificans. Repeated attempts have been made to determine the source of the organism with the point in mind that possibly contamination in the cannery could be prevented. If the source of contamination were restricted to the sugar used in canning or to certain types of soil, the probability of avoiding contamination by suitable precautions would be enhanced.

Numerous samples of sugar, soil and manure were collected and experiments run to determine whether or not the sulphur stinker organism was present. The difficulty of always isolating *Cl. nigrificans* from a sample of material is to be appreciated. Each sample was suspended in distilled water and boiled for four hours in order to reduce the number of other organisms present. Heavy inoculations of each sample were then made into pea medium and beef heart infusion agar. The latter was drawn into capillary tubes, allowed to cool and incubated at 55° C. for long periods. Blackened colonies were isolated and their identity determined. *Clostridium nigrificans* has been in this manner isolated from field soil and horse manure, but its presence is either exceedingly rare or the technic of isolation faulty, since three isolations have been made out of 60 attempts, 32 on soil, 24 on manure and 14 samples of sugar. The organism was not isolated from any of the samples of sugar, although it is possible that had further examinations been made isolation of the organism would have been successful.

SUMMARY

Our studies have shown that a thermophilic, sporulating, heat resistant anaerobe is responsible for the blackening occurring in canned sweet corn known as sulphur stinker spoilage. A study of the cultural and physiological characteristics of the organism indicates that it is an undescribed form to which the name *Clostridium nigrificans* has been proposed.

The ability of the organism to withstand the effects of heating makes processing ineffective in its control. We must therefore look to other methods. The fact that the organism is a thermophile offers the possibility of control by adequate cooling methods. Whether control of sulphur stinker spoilage by cooling would be commercially feasible will have to be determined. Another suggested method of control lies in the prevention of contamination in the plant by avoiding the use of raw materials harboring the organism and by locating any foci of infection within the plant and the elimination of these foci.

The effect of *Clostridium nigrificans* in causing spoilage in various canned vegetables has been pointed out and its importance in the case of peas has been emphasized.

Further studies on the source of the contamination are desirable in view of the possibility of avoiding bringing the organism into the cannery.

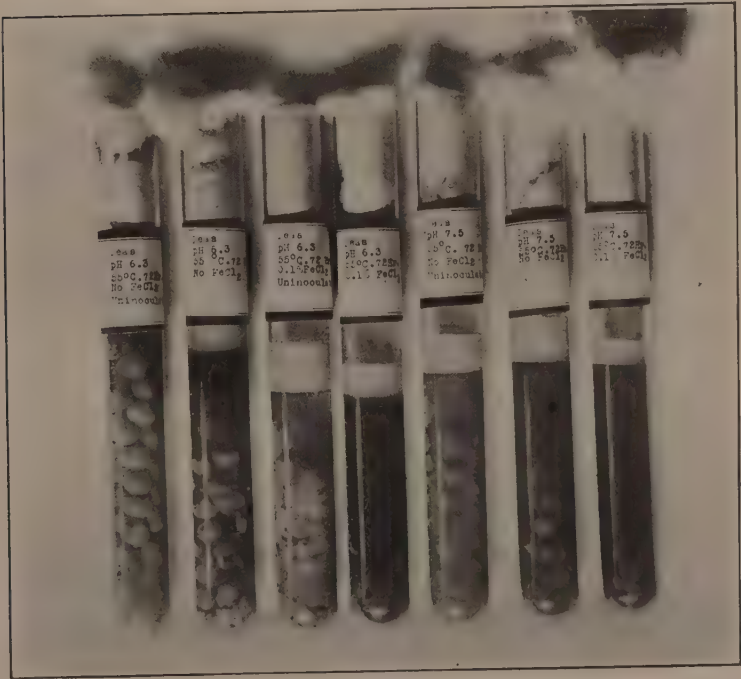
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PLATE I

Fig. 1.. Sulphur stinker spoilage in "Maine style" canned sweet corn.

Fig. 2. Effect of the sulphur stinker organism on canned peas.



MATHEMATICAL LOGIC

By J. S. TURNER

From the Department of Mathematics, Iowa State College.

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Logic is the science of those laws of thought which apply to propositions; it is a system of rules for testing and correcting thought, rather than a description of the processes of thought.

The science was created by Aristotle (384-322 B. C.); it has developed along scholastic lines, with scarcely any essential changes. The essential feature of the scholastic method is this: Something, let us say X , is introduced for discussion; various kinds of X are named and defined, but the meaning of X is not indicated. This scholastic logic is ill-adapted to mathematical requirements.

Mathematical logicians have tabulated the forms of reasoning used in mathematics, and they have demonstrated them from a few principles; by these means they have explained many logical difficulties. But in the treatment of the principles, the scholastic method is retained. We are told that " p denotes an elementary proposition", and " p is true or not true", thus there are several kinds of propositions; but we are not told the meaning of "proposition".

There must be undefined terms, but there is no reason why there should be undefined *logical* terms. There are fundamental ideas which we all possess. We can indicate the terms which denote these ideas by suggestive descriptions. We can select these terms, whose meanings we know, as our undefined terms; by means of them we can define all technical terms, including logical terms.

The first section of this monograph describes the scope and explains the meaning of logic and mathematics. Sections II and III describe certain fundamental instinctive ideas, and show how other instinctive ideas are formed from them. Section IV describes the processes of definition and demonstration. Sections V and VI contain definitions of logical terms, and demonstrations of logical theorems.

The reasoning used in mathematics seldom involves more than the simple processes tabulated in section IV, and the theorems in sections V and VI. If this reasoning is to be expressed by formal logical theorems, further theorems are frequently required; these are to be found in other* works.

A few articles, marked with an asterisk, may be deferred to a later reading.

I. INTRODUCTION.

1. **Common Sense.** I am a being, or ego, capable of receiving sensations, impressions, or stimuli, and of forming mental pictures, conceptions, or ideas, as a result of these sensations. I am situated in a body containing sense organs each capable of receiving a different sensation,

*E. g. Whitehead and Russell, *Principia Mathematica*.

and am surrounded by existences, bodies, or natural objects, which cause these sensations.

I can control my body by what I call the exercise of will, and can change my position so that I become surrounded by different objects; but I cannot so control surrounding objects.

I can communicate my ideas to certain bodies which resemble my own, by means of language; and these bodies can communicate ideas to me. These ideas, I find, closely resemble mine, and I conclude that the bodies contain egos like myself. Such egos call themselves human beings, or persons.

I also find that the sensations received by other persons closely resemble mine, and I presume that the *same* existences which cause my sensations also cause theirs. We call these existences, taken as a whole, The World, or Nature.

We call any group of sensations, with the associated ideas, an experience, or fact. We are able to remember our experiences, *i. e.*, we have the faculty of reconstructing in the mind our ideas of experiences; and we call these memories, taken as a whole, Experience. We cannot reconstruct sensations voluntarily, as we do ideas.

When we receive impressions, we add to them certain ideas derived from experience; we obtain a perception of objects from a group of sensations, by adding separation, place, time, and other ideas. The added ideas react upon and quicken our sensations.

We consider that those objects which produce any particular sensation have some characteristic or property in common, which underlies or causes this sensation. Thus we say that chalk, snow and sugar possess the property of whiteness; and, for want of separate words, we say that they cause the sensation of whiteness, and that this sensation causes the idea of whiteness.

In recalling the idea of a property, the mind forms the picture of an object which possesses the property in question, but which is extremely indefinite in other respects; such a picture is called an abstract idea. We employ abstract ideas in constructing new ideas, *i. e.* ideas which are not memories of experiences. This creation of ideas is called Thought.

2. Knowledge. Though we cannot control objects as we do our own bodies, we can use our bodies to change the positions, shapes, *etc.* of objects; in other words, we can "do things", or "take action". Many diverse actions are necessary to sustain the bodily existence which we call life.

We often wish to know the consequences of an action without actually experiencing them. Now it is found that these consequences do not depend upon the time and place of the action, or on the person who performs it, but only upon the action itself, and the objects upon which it is exerted; hence, if we do not know them from our own experience, we may learn them from the experience of others.

Knowledge of this experience is so useful that it has been collected in books. The amount of this printed knowledge is now so great that it must be arranged in an extremely methodical and compact form to facilitate reference; Logic and Mathematics play an important part in this arrangement.

3. Categorical Logic. We can separate in our thoughts objects which possess a given property, from those which do not possess it. These objects are said to form a class, or category; thus we have the class of white objects, the class of living beings. All objects which do *not* possess the given property constitute the *complementary* class.

We can separate all objects in a given class, into a sub-class containing all objects of that class which possess a second property, and the complementary sub-class containing all objects of the class which do *not* possess this second property. Thus we can separate the class of living beings into plants and animals.

Similarly many ideas have properties, and can be separated into classes and sub-classes.

A *unit* is one object, or one idea.

A *primary* or *categorical* proposition is a statement that some units of a certain class are contained in another class; ("some" denotes *one* or *more*, perhaps *all*). An example is "The earth is a planet"; "The earth" and "a planet" are the *terms*; "The earth" is the **subject*; and "is a planet" is the *predicate*.

The *Universe* is the class which contains every unit.

In certain cases, the truth of a categorical proposition may be in doubt; in such cases, we can test its truth by means of certain principles and processes which are derived from common sense. The discussion of these principles and processes constitutes *Categorical Logic*.

The chief principle is Aristotle's *Principle of Contradiction* (his *Dictum de omni et nullo*), viz. "It is impossible for anything to possess a property, and also not to possess it." It follows that no unit can belong to a class, and also to the complementary class.

The chief process is the *sylogism*. A syllogism consists of three propositions, such that the third is necessarily true if the first two are true. An example is: "The earth is a planet; all planets move; therefore the earth moves." Here "The earth is a planet" is the *major premise* (or premiss), "all planets move" is the *minor premise*, and "the earth moves" is the *conclusion*.

One method of showing that a categorical proposition is true is to exhibit it as the conclusion of a syllogism whose premises are true; other methods will be pointed out later.

4. Hypothetical Logic. A *conditional proposition* is one which may be true (*i. e.* in accordance with observation or conception) at one time and place, and false at another time and place; an example is "The sun is eclipsed". At a given time and place, such a proposition must be either true or false.

A *secondary*, or *hypothetical*, proposition is a statement that the truth of one conditional proposition is always associated with the truth of another; such a proposition is also called an *implication*. An example is: "If the sun is totally eclipsed, the stars appear". The first proposition, the truth of which is presumed (the sun is totally eclipsed), is called the

*Sometimes, the subject is considered to be that which the grammatical subject denotes; and the predicate, the state or action which the grammatical predicate denotes.

hypothesis; the proposition whose truth is implied (the stars appear) is called the *conclusion*.

Hypothetical, or *conditional*, logic investigates the truth or falsity of implications, by means of principles and processes which are derived from common sense, and which are analogous to those of categorical logic. Thus the Principle of the Excluded Middle (or, Excluded Third) is "It is impossible for a conditional proposition to be true, and, at the same time and place, to be false". An example of a hypothetical syllogism in scholastic logic is: "If the sun is totally eclipsed, the stars appear; but the sun *is* totally eclipsed; therefore the stars appear". In Mathematics, a different kind of hypothetical syllogism is regularly used, illustrated by: "If the sun is totally eclipsed, the stars appear; if the stars appear, the earth receives some light; therefore if the sun is totally eclipsed, the earth receives some light". In each case, the first two propositions are the *premises*, and the third is the *conclusion*.

The discussion of hypothetical logic is continued in section VI.

5. Logical Reasoning. In common sense reasoning, we often encounter opinions and ideas which are contradictory. We can remove most, if not all, of these contradictions, by securing agreement on the meaning of terms and on the truth of propositions. For this purpose terms are defined, and propositions are demonstrated.

A *definition* of a word or phrase is a statement of its whole meaning; this statement must consist of words or phrases which have already been defined, or are assumed to be generally understood. Since the succession of definitions cannot be unending, there must be undefined terms. These terms represent fundamental ideas, such as "existence", "separation"; and all we can do toward explaining them is to give synonyms, and examples of their use.

A definition can always be put in the form of a categorical proposition whose subject is the term to be defined; *e. g.* "Common sense is that judgment in regard to first principles in which all men, in general, agree." When a definition is thus expressed, the converse of the proposition is true; *e. g.* "That judgment in regard to first principles in which all men, in general, agree, is common sense." Thus a definition may be regarded as two postulates (see below).

In its simplest form, a *demonstration*, or *proof*, of a proposition is a syllogism whose conclusion is that proposition, and whose premises are either demonstrated propositions, or unproved propositions whose truth is asserted. But a demonstration is usually a succession of such syllogisms, the proposition to be proved being the final conclusion. The propositions in a demonstration may be categorical propositions, in which case the demonstration is essentially a definition; but they are usually implications.

Since the succession of syllogisms cannot be unending, there must be unproved propositions; these are called *postulates*. A postulate is called an *axiom* if it is a part of common knowledge, *e. g.* "The halves of equal things are equal"; it is called a *law*, or *principle* if it is derived from experiment and logical reasoning.

A different kind of demonstration, called *reductio ad absurdum*, will be described in section VI.

When a branch of knowledge is entirely demonstrated from postu-

lates, any contradictions which occur must arise from the postulates. If the postulates lead to a contradiction, they are said to be *inconsistent*; and they must be changed so that no contradiction arises, *i. e.* they must be made consistent.

To illustrate the removal of an inconsistency by logical reasoning, consider the question "What will happen if an irresistible force meets an immovable object?" An inconsistent answer is that the immovable object will be moved. The logical answer is that an irresistible force is one which no object can withstand; therefore if an irresistible force exists, an immovable object is impossible.

It will be seen that logical reasoning improves common sense by removing inconsistencies, but that it is itself founded upon common sense, and therefore has no greater validity than that part of common sense which is free from inconsistencies.

6. Philosophy. Philosophy may be described as the effort to extend and improve common sense by logical reasoning.

Common sense is always growing; we learn by experience. A new idea is at first shadowy; the only tests of its soundness are personal opinion, and the absence of contradictions with other ideas. It takes definite shape and gains general acceptance, *i. e.* inclusion in common sense, only after much discussion and thought.

It is natural, then, that philosophers should differ among themselves. There are two main divisions of philosophers: the *realists*, who assert that external objects really exist, and that differences in sense impressions are due to real differences in objects; and the *idealists*, who assert that sensations and ideas are the only existences.

It seems strange, however, that *reality* should be emphasized. A philosopher is concerned with logical descriptions and explanations of phenomena; and their actual existence is merely a postulate which is irrelevant to these explanations. The difference between the two schools is wider than this. The realist spends much of his thought upon external objects; he meets with great difficulties, and his writings are often obscure. The idealist endeavors to explain all that he is conscious of, without any reference to external objects; he is generally more successful, or at least more intelligible, than the realist. But philosophy is constantly progressing, hence a criticism may not be permanent.

The following *extracts present some idealistic opinions which are, or may soon become, common sense ideas:

"An individual is not characterized by any sameness in the thing-in-itself, but by the sameness in, or permanency of, a certain group of sense impressions; this is the basis of our identification".

"Whenever a sequence of perceptions *D, E, F, G* is invariably preceded by the perception *C, . . . C* is said to be a *cause* of *D, E, F, G*, which are then described as its *effects*. No phenomenon or stage in a sequence has only one cause, all antecedent stages are successive causes, and as Science has no reason to infer a first cause, the succession of causes must be carried back to the limit of existing knowledge,"

"Space is an order or mode of perceiving objects, but it has no existence if the objects are withdrawn,"

*Karl Pearson, *The Grammar of Science*.

"The space of our perceptions is finite, and varies from individual to individual with the range and complexity of his perceptions. As it is just large enough for the perception of phenomena, so it is just small enough, by which we are to understand that it is not 'infinitely divisible'. The limit to its divisibility is the limit to our power of perceiving things apart. . . . We may possibly *conceive* smaller divisions, but in doing this we have passed from . . . the space of perception to the space of geometry."

"We are not compelled to postulate a space outside self for phenomena, and spaces inside for memory thought and the psychical processes, but rather we must hold that the mode in which we perceive in these different fields is essentially the same, and that this mode is what we call space."

"As space is one mode in which the perceptive faculty distinguishes external objects, so time is a second mode, As space marks the coexistence of perceptions at an instant of time, so time marks the succession of perceptions at a position of space."

"When I can no longer carry back the sequence of phenomena, there time ceases for me because I no longer require it to distinguish an order of events."

7. Science. Science is knowledge reduced to system. Scientists collect and classify facts, which are discovered by observation and experiment. They then endeavor to find postulates, or hypotheses, from which these facts can be logically deduced. They draw further inferences from these hypotheses, and test them by additional experiments. If these inferences are verified in each of a large number of cases, the hypotheses are said to be established. An established hypothesis is called a *principle*, or *natural law*.

Their object in this procedure is to secure economy of effort; the laws can be memorized, and the methods of drawing inferences can be learned. Knowledge in this form is more convenient than a large collection of isolated facts.

Some branches of knowledge have only reached the preliminary stage of collecting and classifying facts; these are called *descriptive sciences*. The branches of knowledge in which principles have been discovered from which many facts are inferred, are called *exact* or *precise sciences*. The sciences are always growing, hence no classification of them can be permanent.

Scientists are always trying to discover more fundamental laws, *i. e.* laws from which a greater number of facts can be deduced. Their ideal is a system of natural laws which will enable them to forecast the course of any possible experience.

Science improves common knowledge by making it conform to natural laws. A reputed fact which is contrary to a natural law is rejected, unless it is attested and verified by competent observers, in which case the law is modified.

Natural laws are a part of common sense, hence Science is entitled to general acceptance; but Science has precisely the same "certainty" as common knowledge which is in accord with observation.

8. Mathematics. There are differences of opinion as to the definition of Mathematics. It is customary to state that it is the science which treats of number and form; and that it is divided into *Pure Mathematics*,

which considers only number and form, and *Applied* or *Mixed Mathematics*, which considers these in connection with other properties, such as mass and motion.

According to what may be called the *scientific* view, Mathematics consists of those sciences and parts of sciences in which all results are demonstrated from principles.

According to mathematical logicians, Mathematics is the demonstration of implications out of given postulates. This view is emphasized in Bertrand Russell's dictum: "Mathematics is the subject in which we never know what we are talking about, nor whether what we say is true."

Each of these views is reflected in various branches of Mathematics. In Arithmetic we have a treatment of number, usually without demonstrations; in Applied Mathematics, emphasis is placed upon facts and formulae; postulational methods are chiefly used in topics where serious logical difficulties have appeared.

A characteristic of Mathematics is the use of *signs*, or symbols, to denote words and operations. The purpose of these signs is to present the subject more simply and clearly; but at first they have the opposite effect, and a great deal of time and effort is expended in learning to use them readily.

Mathematical logicians are continually trying to detect and remove illogical elements in reasoning; to discover new methods of demonstration; and to extend the scope of known demonstrations by enlarging or otherwise changing the meaning of the terms, in such a way that the demonstrations will remain logically true. Pure mathematicians are chiefly concerned with the discovery and demonstration of properties of numbers and geometric forms. Applied mathematicians are continually extending the applications of Pure Mathematics to other branches of Science.

9. Mathematical Logic. The reasoning which is based upon conventional logic enables us to remove most of the contradictions which arise in common sense reasoning; but it fails to remove the various differences of opinion which we have noticed, and there are certain contradictions which it does not explain: *e. g.* "Hephaestus the Cretan said 'All Cretans are liars'"; (from which we conclude that "All Cretans are liars" is true, and also false; see §41).

These contradictions and differences of opinion arise from uncertainty as to the meaning of terms, and from postulates upon which there is no general agreement. Thus the principles of logic are expressed by means of terms which are not explained, *e. g.* separation, class, things, time; consequently there is a lack of complete agreement as to the meaning of these terms and the principles themselves.

Various systems of Mathematical Logic secure a greater degree of agreement by revising the foundations of conventional logic. Now there is an instinctive reasoning which underlies conventional logic, and appears to be the same for all human beings; there are certain fundamental ideas which we all possess, and there is the principle that there must be no inconsistencies. Our purpose is to explain this instinctive reasoning, and to use it as the basis of a system of Mathematical Logic.

II. MATHEMATICAL PHILOSOPHY

10. Method. In this section and the next, we examine certain ideas which are usually regarded as fundamental. It is found that many of these ideas can be explained in terms of the rest, by means of definitions and postulates. Informal descriptions of the remaining fundamental ideas are given. Since all of the ideas discussed enter into the structure of language, these descriptions frequently employ ideas which are explained later.

This examination is not merely a description of instinctive reasoning, for the latter, though reliable in familiar situations, repeatedly leads to inconsistencies in abstract statements. It is a description of instinctive reasoning from which all observed inconsistencies have been removed; hence it is a species of Philosophy. Since its purpose is to improve the foundations of Mathematics, we call it Mathematical Philosophy.

The examination is incomplete, for it is restricted to ideas (and existences) which enter into Logic, and not all of these are examined. Moreover to be complete it must reduce the number of fundamental ideas to the smallest possible number, and be entirely free from inconsistencies. There is no means of proving completeness in these respects.

In the present section, certain ideas (and existences) are discussed which are usually neither defined nor explained. The treatment of these ideas which is adopted in this monograph is explained as precisely as possible. In the following section, many ideas are defined of which imperfect explanations are usually given.

11. Some Fundamental Ideas. There are certain ideas which appear to be possessed instinctively by everybody. The following remarks do not *explain* these ideas, but they may help the reader to *recognize* them; *i. e.* to discover, in each case, which of these ideas is denoted by a certain word or phrase.

Let us begin with the statement "I am". I possess a certain state or condition which I call *consciousness*, and a certain mental faculty which I call *attention*. I can direct attention to myself, or elsewhere. When I direct it to myself, I say "I am".

"I" is the subject of this sentence; it is a word which denotes an *existence*. It indicates a particular kind of existence, a person, but here we attend to the general idea.

"am" is the verb; it is a word which indicates that an act of attention is now followed by a perception. Its form, or inflection, indicates first person, singular number and present tense; these may be disregarded here, since separation, person, number and time are discussed later.

If I direct my attention elsewhere, I may become conscious of something, or not. In the former case I say "something is there", in the latter, "nothing is there". But in the former case my thought would better be represented by "I am conscious of something there, and perhaps I can become more fully conscious of it"; and in the latter case by "I am not conscious of anything there, and it is useless to continue the act of attention".

In the sentence "something is there", "something" is the subject; it represents an *idea*, or *existence*: "is" is the verb; for comment, see "am"; "there" is a word which indicates the direction or place of the act of at-

tention, and therefore limits (modifies) "is"; direction and place are discussed later.

In the sentence "nothing is there", "nothing" is the subject; it is a word which is used when some act of attention has failed; it does not represent an existence.

We proceed to develop these remarks into postulates. In the first two postulates, "subject" and "predicate" have the meaning stated in the footnote to §3.

I. *An existence* is the most comprehensive (*i. e.* least restricted) subject. (Every grammatical subject, except *nothing* and its synonyms, denotes an existence.)

An indicates singular number, but for the present this limitation is disregarded. Synonyms for "an existence" are *anything, something*.

II. *Exists* is the most comprehensive predicate.

The inflection indicates singular number and present tense, but here the number and tense are disregarded. Synonyms for "exists" are *is, is in existence*.

III. *An existence exists*.

IV. *Nothing* does not exist.

The sentence "nothing is there" appears to be a contradiction of IV, but it does not denote an inconsistency; see section IV.

Consciousness usually involves sensations and perceptions; synonyms are *immediate knowledge, awareness*.

Attention is directed consciousness; synonyms are *regard, particular notice*.

Whatever we are conscious of, exists; but it is neither affirmed nor denied that whatever no one is conscious of, does not exist.

"An existence," "consciousness", "attention", are fundamental existences. (See §§35, 36.)

12. Separation and Connection. Separation is usually performed instinctively; to direct our attention to it, let us consider some examples:

Perception of separation; I attribute sensations to something which is not myself; I perceive that "that which causes sensations" is separate from myself. *Concept* of separation; I regard "me" and "not me" as separate. *Act* of separation; I can separate an orange into slices. *Faculty* of separation; I can change an indefinite perception into a perception of objects. This separation is usually influenced by experience; when I see a shelf of books, I infer from experience that the books are separate objects. *Absence* of separation; occasionally, in strange surroundings, I feel bewildered through failing to recognize individual objects.

Connection is the contrary of separation.

Let us consider the postulate "A separation changes an existence into several (*i. e.* separate) existences". This postulate has no meaning unless we can distinguish between "an existence" and "several existences". To make this distinction we admit the following fundamental existences:

External separation; *i. e.* separation from other existences.

External connection; *i. e.* connection with other existences.

Internal separation; *i. e.* separation into other existences.

Internal connection; *i. e.* no separation into other existences.

We postulate that these existences are distinct.

We also consider particular kinds of existences. "Being" and "entity" are usually regarded as synonyms for existence; here we assign special meanings to them.

Def. 1. A being is externally separated (*E.g.* a cloud).

Def. 2. An entity is internally connected. (*E.g.* a mountain.)

We could also introduce terms to denote "an existence which is externally connected" and "an existence which is internally separated"; but we do not require precisely these ideas; *part* and *class*, which are discussed later, are modifications of them.

Existence is simply that which exists. It does not involve the idea of separation. It may be separated into other existences, or not; it may be connected with other existences, or not. Internally, a *being* may be either separated or connected; externally, an *entity* may be either separated or connected.

Now external separation will not enable us to distinguish between "a being", and "several beings"; a being which is internally separated is several beings. The distinction cannot be made unless the being is internally connected. Similarly internal connection will not enable us to distinguish between "an entity", and "entities"; the distinction cannot be made unless the entities are externally separated. Hence the only cases of the proposed postulate which have both meaning and utility are:

V. Internal separation changes an entity into several beings.

VI. External connection changes several beings into an entity.

Def. 3. A unit is an existence which is internally connected, and externally separated.

Thus a unit is both a being and an entity.

When particular attention is paid to a being or an entity, it is frequently converted into a unit. Thus if I try to estimate the height of a mountain, I usually think of it as separated from the rest of the earth by a conceptual surface.

Va. Internal separation changes a unit into several units.

VIa. External connection changes several units into a unit.

13. Percepts, Sensates and Concepts. We distinguish whatever we are conscious of, into sensations and conceptions. We distinguish sensations according to the organs through which they are sensed, and we distinguish conceptions into ideas of sensations, and ideas created by thought (see §1). Perceptions are sensations to which remembered ideas are added.

Now perceptions, sensations and conceptions involve time and other ideas, hence we cannot regard them as fundamental. We therefore introduce percepts, sensates and concepts.

A *percept* is perceived. It differs from a perception in that it does not involve the idea of time; if an orange is perceived yesterday and today, there are two perceptions, but only one percept. A *perception* cannot exist apart from sense organs and mental faculties; but it is neither denied nor affirmed that a *percept* cannot exist apart from them. A percept is remembered.

A *sensate* is "sensed" (*i. e.* known by an impression on a sense organ). It differs from a sensation in that it does not involve the idea of time. It differs from a percept in that no ideas derived from experience or mental

faculties (except distinction from other sensates, and absence of distinction *into* sensates) are added. It is neither affirmed nor denied that a sensate cannot exist apart from sense impressions. A sensate is remembered.

Thus "redness" is a sensate, and no assertion is made about it except that it is sensed and remembered, that it is not resolved into sensates, and that it is distinguished from all other sensates, *e. g.* whiteness, sound, weight.

We shall use "concept" to denote the idea (mental picture) of a percept, sensate, or mental faculty; and "conception" to denote any other mental picture, *e. g.* an atom, a fairy. A concept satisfies all the definitions and postulates of the original existence, except that it is conceived; the statement of the definitions and postulates for particular concepts will therefore be omitted.

VII. Whatever I am conscious of, is a sensate, or a concept, or a combination of these.

VIII. A unit is a percept or a concept.

A perception always involves a sensation, and usually involves different sensations; hence we shall say that:

IX. A percept *possesses* a sensate, and may possess distinct sensates.

For example, I can see the shape and color of a book, and I can feel its weight; so I say that the book possesses the sensates form, color and weight.

14. Distinction. If several beings possessed an internally connected sensate, it is difficult to avoid the conclusion that the beings would be internally connected; and if an entity possessed externally separated sensates, it would seem that the entity must be internally separated. Thus if a ball is white and round, and if whiteness is externally separated from roundness, it would seem that the ball must be several beings.

We avoid this difficulty by postulating that sensates and concepts of sensates are *distinguished*, and not separated. Thus we have the fundamental existences *external distinction* and *indistinction*, and *internal distinction* and *indistinction*.

Def. An *essence* is externally distinguished.

Def. A *substance* is internally indistinguished (*i. e.* unresolved).

Post. Internal distinction changes a substance into distinct essences.

Post. External indistinction changes distinct essences into a substance.

However, substances and essences, as here defined, are logical abstractions; practically, we may identify an essence and a substance with a *monad*. Moreover, the distinction of sensates depends upon acuteness of perception, and is seldom reversed.

Def. 4. A *monad* is externally distinguished, and internally unresolved.

X. Internal distinction changes a monad into distinct monads.

XI. External indistinction changes distinct monads into a monad.

XII. A sensate is a monad.

A monad is not a unit, but it can be converted into (regarded as) a unit by being thought of as externally separated and internally connected.

Separation is closely associated with the faculties of perceiving space and time. Distinction is closely associated with immediate sense impressions.

***15. General Classes.** It is not easy to frame a definition from which all the statements made about classes can be demonstrated.

First, a class *contains*. This implies that it is an existence which is internally separated. The existences which it contains are externally separated, hence they are beings. The class may be

(A) externally separated, or (B) externally connected;
a contained being may be

(C) internally separated, or (D) internally connected.

Secondly, we know whether beings are, or are not, contained in a class. If the class is perceived, this knowledge is obtained from sensates; the sensates must be possessed by the beings, or the class, or both. If the class is conceived, the knowledge is obtained from concepts of sensates.

Thirdly, it is supposed that a class can be distinguished from a being which it contains. This distinction can only be made by means of sensates (or concepts of sensates), and the percepts (or concepts) (A), (B), (C), (D).

Fourthly, there are classes which contain classes.

Now suppose that a class is not externally separated; then it is the Universe. Each of the above requirements is satisfied, except that there is no class of classes.

Next suppose that a class is externally separated, and that every contained being is internally connected. Then the class is a being, and the contained beings are units. Each of the above requirements is satisfied, except the fourth; there is a contradiction between "a class contains only units", and "a class contains classes".

Finally, suppose that a class is externally separated, and that some contained being is not internally connected. Then this being, like the class, is externally separated and internally separated. There is no definition or postulate which provides a distinction between the class and the contained being; if they possess the same sensates, there is no means whatever of distinguishing them. "Class" and "contained being" are alternative terms. This conception of class is associated with fallacies which arise from the supposition that the class can be distinguished from such a contained being.

We can introduce a postulate to exclude the possibility that a class may contain a being which possesses exactly the same sensates as the class itself. A class which is defined by the following postulates fulfills each of the above requirements:

(I) A class is a being, or several (*i. e.* separated) beings.

(II) There is a means of knowing (*i. e.* a criterion) whether any particular being is, or is not, a being of the class.

(III) This criterion is a sensate (or sensates) possessed by the beings of the class, or the class, or both.

(IV) The criterion is not identical with all the sensates possessed by a being of the class.

Def. A being which is a being of the class is *contained* in the class. A being which is not a being of the class is *not contained* in the class.

However, there are other requirements; *e. g.*

(R) If A contains B and B contains C, then A contains C.

(R) implicitly contains the first four requirements, from which (I),

. . . , (IV) were derived. Evidently, (*R*) cannot be demonstrated from (I), . . . , (IV); to prove that *A* contains *C* we require that *A* can be distinguished from *C*, and from every being contained in *C*.

If we replace (III) and (IV) by

(III') This criterion is a sensate (or sensates) possessed by each of the beings of the class,

(IV') The criterion distinguishes the class from every being except itself,

we can prove (*R*), the demonstration being similar to that of §45.

Now (I), (II), (III), (IV) do not provide an adequate definition of a class, because we cannot demonstrate (*R*) from them. Also (I), (II), (III'), (IV') define only a restricted type of class; for (III') excludes numbers, which satisfy (*R*).

In conclusion, we have derived a comprehensive definition of a class from the statement "*A* contains *B*", where *A* and *B* are distinct; we shall call an existence which satisfies this definition a *general class*. We reject the case in which *A* is the same as *B* because inconsistencies may creep in if there are distinct terms, not defined to be equivalent, to denote the same existence. We have not found a definition, from which (*R*) can be demonstrated, which includes all general classes for which (*R*) is true.

16. Classes. We shall define a class by the following postulates:

XIII. A class is several (*i. e.* separated) units.

XIV. There is a means of knowing (*i. e.* a **criterion**) whether any particular unit is, or is not, a unit of the class.

"Two" is a class, for it is several (*viz.* two) units, and the criterion is simply that we perceive, or imagine, the units. The ocean is not a class of drops of water, since the drops are not separated. The winning tickets in a lottery do not form a class before the "draw" takes place, because there is no means of knowing that they are winning tickets. A class is usually a number of things, and the criterion is usually a property, or several properties.

This definition does not exclude the possibility of a class of classes. To see this, consider the classes "all white cats", and "all white cats with blue eyes". The latter class satisfies the criterion "white cat", but since it is not a unit we make no assertion as to whether it is contained in the former class, or not. A class cannot be identical with a contained unit; for the class is not a unit.

Def. 5. A unit of a class is **contained** in the class. A unit which is not a unit of a class is **not contained** in the class.

A unit, with a criterion, can enter into processes which involve classes, as if it were a class. Such a unit is called a *unit class*; it is defined by XIII and XIV, except that "several units" is replaced by "a unit". The criterion of a unit class should not be identical with all the sensates possessed by the unit, for the class would then be identical with its unit.

Def. 6. The class which contains every unit is called the **universal class**.

This is the only class which is not externally separated.

Def. 7. A **set** is a class in which the criterion is simply that the units are perceived (or conceived).

17. Class-units.

Def. 8. A class-unit is a unit which is derived from a class by conceptually connecting its units.

A unit class is a class-unit.

Def. 9. A class-concept is a class-unit which is *conceived*; a class-percept is a class-unit which is *perceived*.

Thus from "a collection of different books" we derive the class-unit *a library*; this is a class-concept if it is conceived, a class-percept if it is perceived. Unless it is a unit class, a class-unit is a construct (§24), not an individual; thus a library is not a book.

The name of a class is frequently identical with that of the corresponding class-unit; *e. g.* in "a hundred is ten tens", "hundred" denotes a class; but in "a thousand is ten hundreds", "hundreds" denotes class-units.

*18. Relations.

Def. 10. A relation is some mode of existence, and some kind of separation or distinction: "(Something) *exists*, in some manner, *separated* (or *distinguished*), in some manner, from (something)". *E. g.* "is near", "occurs before".

A description of the manner in which existences are separated or distinct, indicates certain ways in which they are connected or indistinguished; thus "occurs before" relates one *event* to other *events*.

"*a* is related to *b*" may be denoted by "*aRb*". The relation *R* indicates certain properties or conditions (§23) of *a* and *b*. If *a* or *b* does not possess these properties or conditions, *aRb* involves an inconsistency.

"Is the same as" is regarded as a relation. It is called the *identical* relation.

Def. 11. A relation is **reciprocal** (or *mutual*) provided that, if *aRb* is true, then *bRa* is also true. *E. g.* "is a neighbor of."

The statement *bRa* must be true for every *a* and *b* for which *aRb* is true. Thus "is a brother of" is not a reciprocal relation.

Def. 12. A relation *R* is **transitive** provided that if *aRb* and *bRc* are true, then *aRc* is true. *E. g.* "occurs after".

R is *intransitive* provided that if *aRb* and *bRc* are true, then *aRc* is *not* true. *E. g.* "is the father of".

If, in *aRb*, we do not wish to express *b*, we may replace the verb of the relation by "possesses", or "has", and the mode of separation, together with *b*, by a *relative state*. Thus "*a* occurs before *b*" may be replaced by "*a* possesses priority". However, *b* is sometimes expressed with relative states, *e. g.* "*a* has priority over *b*". If we do not wish to express *a* we may, in some cases, change "exists" into "has" and replace the mode of separation, together with *a*, by a *relative being*. Thus "*a* is a brother of *b*" may be replaced by "*b* has a brother". But *a* is sometimes expressed with relative beings, *e. g.* "Jane has a brother, John". Relative states and relative beings are not relations in the sense defined above.

19. **Intervals.** We distinguish sensates into sights, sounds, flavors, *etc.* We further distinguish sights into distances and colors; sounds, into noises and notes.

Now we perceive certain similarities in colors. We distinguish them into red, yellow, blue, *etc.*, and we perceive greater similarities between

various yellows than between red and yellow. Thus we distinguish *intervals* between colors, and we can distinguish between a large interval and a small interval. To some extent, we can also distinguish *equal* intervals; *e. g.* that the interval between orange and yellow is about equal to that between yellow and a certain shade of green.

It is therefore possible to select certain standard colors, and to relate any other color to them by noting its intervals from them; this device helps us to remember unusual colors. Moreover, people can agree upon a set of standard colors which will be the same for all; and it becomes possible to describe any color so that any normal person can recognize it. A standard color is a "monad", since it is distinguished from other colors and from all other sensates, and is not resolved into colors; hence a standard color can be regarded as a unit, and a set of standard colors can be regarded as a class.

What has been stated of colors also applies to other sensates.

XV. An *interval* is a distinction, or a separation.

Def. 15. A *scale* is a set (or class) of similar sensates, to which other similar sensates are related by means of intervals.

Def. 16. The *relation* of a sensate to a scale is ("is" and) the set of intervals between the sensate and the standard sensates; or a sufficient number of these intervals to determine the rest.

A *large* interval is one which can be distinguished easily; a *small* interval is one which is distinguished with difficulty; *equal* intervals are those which are distinguished with equal difficulty. These terms imply a reference to the method of estimating or measuring the intervals. We can assert that two intervals are exactly equal only when the equality is a consequence of definitions or postulates.

XVI. Let (AB) denote the interval between the sensates A and B . Then if (AB) and (BC) are both small, (AC) is small, or moderate. Also if (AB) is small and (BC) is large, (AC) is moderate, or large.

III. MATHEMATICAL PHILOSOPHY (Continued)

20. Change and Time. We can hardly imagine a state of affairs from which *change* (alteration, transition) is entirely absent. Life is impossible without physical changes, and thought is impossible without changes in ideas.

We can think of changes of number, form, position, *etc.*, but not of change itself. We try to represent all kinds of change by *motion*, which is change of position. A change is something which enables us to distinguish a perception from a percept.

Although we experience so much change in ourselves, our prevailing perception is *persistence*, which is the absence of change. Our vital changes are, normally, almost unnoticed; most objects appear at rest. Hence the totality of our perceptions may be described as persistences, separated by occasional changes. We try to represent persistence by *rest*, which is the absence of motion.

We recognize a *succession* in our perceptions of rest and motion; rest, then certain motions, then rest, then other motions, and so on. The power of the mind to recognize this succession is called the *time faculty*. Our memories retain, to a considerable extent, not only our perceptions, but also

their order; thus the time faculty also operates in memory. Objects which appear in the same perception are said to appear at the *same time*. Objects *persist*, i. e. they appear in a succession of perceptions, but the successive perceptions may differ in certain respects.

Life processes, such as acts of attention, sleeping and waking, provide us with a succession of changes and persistences to which our perceptions are related; this succession may be called *personal time*. A succession of many changes and persistences is a *long* time; of few, a *short* time. The perceptions of an object can be related to personal time, by noting that they last for a long time, or a short time; or that they occur at the same time, as a meal, *etc.*

When people compare experiences, they often find that their personal times disagree. Hence, instead of these, they use a succession of changes and persistences which is the same for all, such as days, months and years; for times shorter than a day, they use hours, minutes and seconds (which are derived from the daily motion of the sun). This succession forms a *natural* time, to which all perceptions can be related.

Def. 15. *Personal time* is a succession of changes and persistences to which the perceptions and ideas of a person are related.

Def. 16. *Natural time* is a succession of changes and persistences chosen by general agreement, to which all perceptions and ideas can be related.

Thus time is a scale.

Def. 17. Each change and persistence in time is called an *instant*. (See remarks following Def. 24.)

XVII. An instant is a unit.

That instant (or *moment*) which is perceived is *present time*, or *now*. Remembered time is *personal past time*; the concept of time derived from historical records is *historical past time*. Anticipated time is *future time*. Only *present time* is *perceived*; past and future time are *conceived*.

Any instant *A* of past time is said to have occurred *before* the present instant *B*, and *B* is said to occur *after A*; *A* and *B* are said to occur at *different* times. If *A* occurs before *C* and *C* before *B*, *C* is said to occur *between A* and *B*. The successive instants which occur between *A* and *B* are called the *period* from *A* to *B*. There are successive periods during which similar sequences of perceptions occur; e. g. days and years; by studying one such period we can foretell, to some extent, the course of events during a recurrence of that period.

Change, and succession, are fundamental existences.

21. Number. When I examine a unit, e. g. an orange, I can fix my attention on the facts that it is externally separated and internally connected, and abstract (i.e. ignore) its other properties.

Def. 18. *One* is a unit from which everything is abstracted except that it is separated externally, and connected internally.

Def. 19. A *number* is *one*, or *several ones*.

Numbers have names, and are denoted by symbols, called numerals; the names of certain numbers, with the corresponding *Arabic* numerals, are as follows:

One is denoted by 1.

Two is one, and one more; it is denoted by 2.

Each of the numbers **three, four, five, six, seven, eight, nine**, is one more than the preceding; they are denoted, respectively, by **3, 4, 5, 6, 7, 8, 9**.

We can recognize certain numbers with a single act of perception. Unless they are arranged in some special manner, we cannot recognize numbers beyond six, or seven, in this way; to recognize nine, we say or think, six and one make seven, and seven and one make eight, eight and one make nine. Hence, as a rule, the idea of *succession* enters into the *recognition* of particular numbers; but this idea does not enter into the *perception* of numbers.

In the succession 1, 2, 3, 4, . . . a number which is written before another is *less* than the other; and one which is written after it is *greater*. This sequence of numbers can be continued indefinitely. Numbers greater than nine are denoted by combinations of the Arabic numerals. Thus

Ten is nine and one more; it is denoted by **10**.

Eleven is ten and one more; it is denoted by **11**.

The numbers of this sequence are *definite* numbers.

There are also *indefinite* numbers. *Few* means less than, and *many* means greater than, some unmentioned number. *Fewer* means less than, and *more* means greater than some specified number. When applied to numbers, *some* means "one or more"; *several* (in this monograph) means "two or more"; *all* means every one of a class; *the rest* means the units not subtracted, or not previously mentioned; *most* means "some, more than the rest", or "the greatest of three or more numbers".

"Two men" is an example of a *concrete number*. Here the process of abstraction has not been carried so far as "two".

A more comprehensive definition of "one" can be given: "*One* is a *being*, or an *entity*, from which every sensate is abstracted except that it can be distinguished from other existences". Hence we obtain more comprehensive definitions of 2, 3, . . .; thus we can speak of "two classes", "two mountains". This extension requires us to verify that the number can be distinguished from an existence which it contains, or in which it is contained; e. g. we should not speak of "two twos", if the "twos" are "two twos"; see §15. The extension is seldom required. If the term "a number of classes" is used, "several classes" or "a number of class-units" will usually express the intended meaning more correctly. In the proposition "two twos make four", the "twos" are class-units.

22. Correspondence.

Def. 20. A **correspondence** is a reciprocal relation between units of one class, and units of another class.

Def. 21. A **one to one** correspondence is a correspondence in which one and only one unit of one class corresponds to one and only one unit of another class.

Def. 22. If a unit *A* and another unit *B* are in one to one correspondence, *A* and *B* are said to form a **couple** (or *pair*).

XVIII. If the classes *A, B*, and also *B, C*, are in one to one correspondence, then *A, C* are in one to one correspondence.

XIX. If *A, B* and *B, C* are couples, then *A, C* is a couple.

Def. 23. A **number-unit** is a unit which is obtained from a number by conceptually connecting its units.

Thus from "two" we obtain a pair or *dyad*; from "three" we obtain a *triad*. A number-unit is a class-unit. The conceptual connection is similar to that of the letters of a printed word (see end of §33). As a rule, a number and the number-unit derived from it are denoted by the same term; the words "two", "three", *etc.*, and the symbols 2, 3, . . . are used to denote number-units, as well as numbers.

Def. 24. If we can show how to assign a one to one correspondence between the units of a class and the number-units denoted by 1, 2, 3, . . . (or these symbols themselves), the class is **denumerable**; otherwise it is **non-denumerable**.

We can show that a class is denumerable by describing a process by which some unit is paired with 1, some other unit with 2, . . . no unit of the class remaining unpaired. If an instant is defined to be a second, or the time of an event, or the part of a second which precedes or follows or lies between two events, the class of instants in a day will be denumerable; for the instants, as they occur, can be paired with 1, 2, 3, . . .

The unit paired with 1 is called the *first*, that paired with 2 is the *second*, that paired with 3 is the *third*, and so on. First, second, third, *etc.*, are called *ordinal* numbers. The process of assigning ordinal numbers to the units of a class is called *ordering* the class. If the process terminates, the class is *finite*; otherwise it is *infinite*.

Def. 25. Two numbers are **equal** if there is a one to one correspondence between their units.

Def. 26. A **definite** class is a class to which no unit is added, and from which no unit is removed.

If a unit is added to, or removed from, a definite class, that definite class is destroyed and a new one is created.

XX. A definite class is finite.

The letters of the word "group" form a definite class; they can be paired with 1, 2, 3, 4, 5 as in the figure. The succession, or sequence, 1, 2, 3, 4, . . . has the property that if it terminates the last symbol denotes the number of symbols; thus since 5 is the last symbol used in ordering g-r-o-u-p, there are five symbols, and consequently (Def. 25) five letters.

If we assume that the ordinal numbers of any two units can be interchanged, we can prove that the number of units in any definite class is the same, no matter how the units are ordered. The process of discovering the number of units in a definite class is called *counting* the class.

23. Properties and Conditions. Our instinctive knowledge of the properties and conditions of percepts is rather intricate.

Def. 27. An **instantaneous** percept is a percept which is perceived only for an instant. *E. g.* a flash of lightning; a collision.

Def. 28. A **persistent** percept is a percept which is perceived at (or during) several instants.

A persistent percept is usually a thing (§25), or a class of things.

Def. 29. The **sensates** of an instantaneous percept are called its **properties**. Likewise those sensates of a persistent percept which can be sensed whenever the percept is perceived, are called its properties.

"Can be sensed" means "is sensed if the attention is properly directed". It means rather more than "exists", and rather less than "is

sensed". It is considered that a property can be sensed by every person with normal senses; *i. e.* every property is *natural* (Def. 48), not merely personal.

Def. 30. A sensate of a persistent percept which can be sensed during at least one instant, and which cannot be sensed during at least one other instant, is called a **condition of the percept**.

Usually, a condition is natural; but some conditions are not natural, being sensed by a limited number of persons. Thus a total eclipse of the sun is perceived by some persons with normal sight, but not by others; hence, if "total eclipse" is regarded as a condition of the sun, it is not a natural, but a *personal* condition.

Extensions or modifications of Defs. 29, 30 are given in §§ 24, 28.

Def. 31. A percept is said to **possess** properties; a persistent percept is said to **be in** (or *possess*) conditions.

Def. 32. An **essential** property of a percept is one which enters into its definition or postulates.

Def. 33. If a persistent percept loses an essential property (*i. e.* if the property can no longer be perceived), the percept is **destroyed** (ceases to exist). If it gains or loses a property which is not essential, it is **changed** (*improved, or damaged*).

Def. 34. A percept in which certain properties are perceived and others ignored (abstracted), is an **abstraction**.

Thus "a red object", "a human being", are abstractions.

Def. 35. A percept from which no sensate is abstracted is **concrete**. *E. g.* this orange; that man.

XXI. In a concrete persistent percept it is possible to sense new sensates (*i. e.* sensates which have not previously been sensed).

Def. 36. A percept is said to be **equal**, or **equivalent**, to another, if every property possessed by either of them is also possessed by the other.

Def. 37. The statement that two persistent percepts are **the same**, or **identical**, means that a persistent percept perceived at one instant, and a persistent percept perceived at another instant, are one persistent percept.

In this connection, "one" and "two" sometimes have the extended meaning described at the end of §21.

It is difficult to define "fact" and "event". We do not assert that the two following postulates include the whole meaning of these terms.

XXII. The perception or conception that a percept possesses a property, or that a persistent percept sometimes possesses a condition, is a **fact**. *E. g.* "a diamond is hard"; "the lightning was brilliant"; "a monkey eats nuts".

XXIII. The perception or conception of "a persistent percept during the continuance of a condition of the percept", or of "a persistent percept during its creation or destruction" is an **event**. *E. g.* "the sun was eclipsed"; "the death of Socrates".

The *concepts* of persistent percepts, properties, conditions, are called persistent concepts, conceived percepts, conceived conditions, respectively.

24. Individuals, Constructs and Parts.

Def. 38. An **individual** is a unit percept which possesses the *property* of being connected internally, and also the property of being separated externally. *E. g.* "myself".

Def. 39. A *construct* is a unit percept which is merely *conceived* to be connected internally, and which possesses the property of being separated externally. *E. g.* "a (printed) word".

Def. 40. A *part* is a unit percept which is merely conceived to be separated externally; and which either possesses the property of being connected internally, or is conceived to be connected internally. *E. g.* a mountain, a syllable.

If an *individual* is separated into parts, these parts will no longer be the individual. But if one of the parts possesses all the essential properties of the original, it is considered to be the original. *E. g.* if a slice is cut from a loaf of bread, we consider the part which remains to be the loaf. The individual is considered to be destroyed if none of the parts possesses all the essential properties.

If a *construct* (*e. g.* a watch) is separated into parts, the parts (collectively) are usually considered to be the construct in a changed condition. If the parts are put together so that they appear to be as they were, the *same* construct is obtained. If new parts are added, or if any parts are removed, the new construct is considered to be the original if it retains all the essential properties of the original and does not acquire new essential properties; but if there is any change in essential properties, it is regarded as a new construct.

A *part* is always part of an individual or construct. It seldom possesses all the essential properties of the original, for in such cases it is usually identified with the original. If a part is actually separated (*i. e.* if its external separation becomes perceived), it is still regarded as a part, if its properties enable us to reconstruct the concept of the original.

In some cases, if a part possesses a property or condition it is considered that the entire unit possesses it; thus if a knife has a sharp edge, we say that the knife is sharp. In other cases, a property or condition of a part is not regarded as a property or condition of the whole. Thus if one blade of my penknife is sharp, and the other blunt, I do not say that the penknife is sharp, but that one blade is sharp. In such cases, we suppose the unit to be separated into parts such that the properties are possessed, or not possessed, by each of them.

The word "part" is sometimes used with a meaning which is different from that described above. We shall use the word "member" to indicate this other meaning.

Def. 41. A *member* is a percept which is externally distinguished, and which either possesses the property of being connected internally, or is conceived to be connected internally. *E. g.* A man's head; a horse's mane; an angle of a triangle.

25. Things. It might be supposed that a "thing" can be distinguished from a "percept" by means of properties or conditions. To show that this is not the case, let us consider two examples.

When I see a rainbow, I "sense" that it has a certain form, and various colors ranging from red outside, to violet inside. The *sensates* "a certain form", "red", violet", *etc.*, are distinguished in the *sensate* "rainbow"; the *percept* "rainbow" may include the ideas that the form is arch-like, that the colors are due to the refraction of light in passing through raindrops, and that I have seen similar rainbows. There is *one* percept

"rainbow"; it may persist for a considerable time, and it is recognized by its sensates. The rainbow is not "a thing".

At intervals of about 76 years, Halley's comet can be seen, on clear nights, during several months. It is recognized by its period, its retrograde path amongst the stars, and its yellowish tail, which attains a length of over 90 degrees. The motion amongst the stars, and the form and color of the tail, are sensates which are distinguished in the *sensate* "Halley's comet"; the *percept* "Halley's comet" includes the idea that it moves in an elliptical orbit. Halley's comet is "a thing".

A thing differs from a sensate or percept in this respect; we do not say what happens to sensates or percepts when they are not perceived; but we make assertions about *things* when they are not perceived.

XXIV. A *thing* is a unit persistent percept.

XXV. A thing may appear more than once, and at each recurrence it is recognized by its sensates.

XXVI. If a thing existed at any instant, it is asserted that it now exists, and that it has existed at every intermediate instant; unless its destruction has been observed, or is presumed for some reason other than non-perception.

It is from *things* that we instinctively derive our ideas of distance and position. Thence we derive the idea that a thing has a definite position at any instant during its existence. We are then led to the assertion that certain existences, previously regarded as nothing more than persistent percepts (*e. g.* comets), are things.

To explain the meaning of distance on the basis of instinctive reasoning, we require some postulate (XXVI) which distinguishes things from persistent percepts and does not involve the idea of distance. Whatever is asserted to be a thing, must satisfy this postulate. All that the argument requires is that there should be *some* postulate which serves this purpose; perhaps a more suitable one could be devised.

That a thing is a *unit* is not necessarily an *essential property*, for its unity may be merely a concept.

26. Distance. Distance is an interval between sensates; but it is something more definite than this. If I perceive an unknown phenomenon at a certain distance, and place my hand upon it, I expect to experience the sensation of touch. A child, seeing a beam of light in a dusty room, is surprised because it cannot feel the beam. If no distance is perceptible between two things, we say that they "touch", or "are in contact"; we do not necessarily imagine that they *feel* each other, but we imagine that a person could feel both of them, at the same time.

XXVII. Distance is an interval between *things*, or between existences which are conceived to be things.

XXVIII. If there is no distance between a person and a thing, the person can feel the thing.

XXIX. If there is no distance between two things, and if a person can reach them, he can feel both of them at the same time.

Having now explained the meaning of distance, we can use distance to explain the meaning of space.

Since there is no postulate which restricts the meaning of "existences", or "feel" (except that "to feel" is "to sense by touch"), the

above postulates give no assistance in demonstrations relating to distances. The postulates for "distance" and "thing" which give assistance in demonstrations, involve the idea of position. We therefore introduce two definitions which have logical significance.

Def. 42. Distance is an interval which is consistent with the definitions and postulates of §27.

Def. 43. Objects are unit percepts (or concepts) which can be related by means of distances.

XXX. A thing is an object.

Def. 44. Two objects are said to be in **contact** when there is no distance between them.

27. Space. I perceive that many things have the property that the distance between any two of them remains the same; *e. g.* the ground, houses, roads, mountains, *etc.* I say that these things are at rest, and that they form a class. At any instant, I can observe the distances of any thing not in the class, from things in the class. If these distances remain the same, I say that the thing is at rest; if they are changing, I say that it is in motion. I call this class of perceived things, possibly including all the things at rest, a *place*. I also form a concept of this place.

When I move away, I observe other things which maintain invariable distances. I say that these form a class of things at rest, and that this new class of perceived things is a place.

I remember the former place, and some intermediate things and distances. Usually, these intermediate distances remain the same, and I imagine a conceptual place which contains the concepts of the separate places and the intermediate things and distances. Whenever I move to a different place, I extend my conceptual place to include the concepts of the separate places and distances between. This conceptual place, together with the place which I now perceive, may be called *personal space*.

When people compare experiences, they usually find that their personal spaces disagree. The lack of agreement arises from differing estimates of distances, and from the circumstance that different people have visited different places. Hence distances are *measured*, and places are *described*; and the measurements and descriptions are printed in atlases, guide-books, *etc.* Thus a space is obtained which is the same for all; this may be called *natural space*.

We can observe the distances between the *parts* of a thing; with the help of these distances, we derive the abstractions *size* and *form*. On account of the size of things, there is an indefiniteness in measurements of distances between them; hence, in measurement, *things* are replaced by *points, lines, etc.* These objects are discussed in works on geometry.

Def. 45. **Personal Space** is a class of objects perceived or conceived by a person, which maintain unchanging distances, and to which all other objects perceived or conceived by the person are related by means of distances.

Def. 46. **Natural Space** is a class of objects selected by general agreement, which maintain unchanging distances, and to which all other objects can be related by means of distances.

Hence space is a scale (§19).

Def. 47. The **position** of an object is a relative state (§18) which is derived from a sufficient number of distances, from the object to objects of the space, to determine all such distances.

XXXI. At least three distances are required to determine the position of an object.

XXXII. An object has a position in space at each instant at which it exists.

XXXIII. An object cannot have different positions at the same instant.

If I press against a thing, I may move it, or change its form; but I cannot place my hand in the same position as the thing. Hence I conclude that

XXXIV. Two *things* cannot have the same position at the same instant.

28. Properties and Conditions of Objects. If I take an object to different places, I do not, as a rule, perceive any change in its sensates; but the following instances show that such changes may occur.

(1) The times of oscillation of the same pendulum are different at different places.

(2) The direction of a magnetic compass changes very slowly at a given place, but it varies greatly at different places.

(3) If the sun is eclipsed at one place, it will at the same time be visible at other places.

Hence it is conceivable, according to Defs. 29 and 30, that a *sensate* may be a property, and also a condition; for it is possible that the *sensate* can be sensed whenever the object is perceived, in some places, but that it cannot be sensed, at certain times, in other places. To prevent this possibility, we replace Def. 29 by:

Def. 29a. A property of an object is a *sensate* which can be sensed whenever and wherever the object is perceived.

If a condition of an object continues for some time, it is necessary to state the place in which the condition is possessed; for it may not be possessed throughout that time if the object moves away from that place. At a given instant, however, an object has a definite position, hence no change in Def. 30 is required.

If we have a persistent percept or concept which is not localized (*e. g.* a class), or a *sensate* which does not depend upon the position of its percept (*e. g.* unity), we do not mention its position. Thus in such cases we do not use Def. 29a.

Some *sensates* depend upon the distance of the observer from the object, the time of day or year, *etc.* In these cases we observe changes in a *set* of objects. To note properties and conditions of one object, we must compare observations made with "the same conditions"; *i. e.* with the same relations of the object to the other objects upon which the *sensates* depend.

29. Persons. I can perceive and form conceptions of time and space without reference to the experience of other people; but I modify my time and space to make them agree with the time and space adopted by other people. This procedure can be reconciled with the opinion that my knowledge is entirely derived through my *sensates* and mental faculties, because my *sensates* and mental faculties, and other people, have not been defined.

It is only necessary to assert certain postulates about persons (*i. e.* people).

XXXV. A person is a thing which moves itself, and other things, as I do. Usually, it can speak and write.

XXXVI. Its actions, including speech and writing, convey ideas to me.

Its form, and many other properties and conditions, are similar to mine. The ideas which it conveys to me are similar to mine, or are such as I can form. These similarities lead me to assert that

XXXVII. It has consciousness, sensations, mental faculties, and thoughts similar to mine; and its actions, including speech and writing, are expressions of its thoughts.

Its reactions lead me to assert that

XXXVIII. My actions, including speech and writing, convey my ideas to it.

XXXIX. The percepts which we all perceive (*i. e.* other persons and myself) are identical; *e. g.* there is one sun, which gives light and warmth to all of us. This requires that we identify certain fundamental existences, *e. g.* separation, succession, distinction, intervals.

XL. We modify our personal spaces and times, so that they agree, and then assert that there is one space, and one time.

Def. 48. To indicate that an existence is said to be the same for all normal persons, we call it a **natural** existence.

Def. 49. The totality of all natural existences is called **Nature** (or "the external world").

Now "a person" is consistent with my description of "a percept". When I say that my sense of redness, for example, is the same as that of some other person, I do not contradict any former statement; I simply add a new postulate. Since these postulates introduce no inconsistencies, they are admissible (§39).

IV. LOGICAL REASONING

30. Method. In their development, philosophy and logic are intermingled. The philosopher accepts certain statements as true, being guided by logical reasoning. The logician applies tests to decide whether statements are true or false. These tests are statements, of special types, which in the first instance—being a part of common sense—are generally admitted to be true; but which are subsequently modified, as a result of philosophical examination. Thus, as a result of certain philosophical ideas being incorporated in common sense, the bases of logic are modified, and in consequence logical reasoning and philosophy are changed. The effort to eliminate all contradictions results in many changes in philosophy and logic alternately, these changes tending to finer distinctions in thought, and more explicit definitions and postulates.

In exposition, when the changes have been effected, there is some advantage in placing philosophy before logic, because it can be expressed in less technical language. Thus we present philosophy in sections II and III, logical reasoning in the present section, and logic in the two following sections.

The argument of this chapter is as follows: There are two types of errors which arise in reasoning; *viz.* those which arise from confused

thought, and those which arise from language which expresses thought in- exactly. The errors can be corrected by exercising greater care than in section I in setting down the types of reasoning which are asserted to be correct, and in defining the processes of definition and demonstration.

31. Inconsistencies. An inconsistency is a confusion of thought; *e. g.* the Irishman's dream that he was walking about, naked, with his hands in his pockets. The following are common types of inconsistencies:

(A) An object which is assumed to be the same at all times and places, and for all people, is also assumed to be different at different times and places, or for different people.

(B) Objects which are assumed to be different at different times and places, or for different people, are assumed to be the same.

(C) A sensate which is sensed by a given person at a given time and place, is assumed *not* to be sensed by that person at that time and place.

(D) A sensate which is not sensed by a given person at a given time and place, is assumed to be sensed by that person at that time and place.

The fundamental principle of logical reasoning is:

XLI. There must be no inconsistencies.

32. Terms and Propositions.

Def. 50. A term is a word or phrase (or symbol) which denotes an existence.

A term is itself an existence, and in certain cases there are terms which denote terms. It is assumed that a term can be distinguished from the existence which it denotes.

Every noun, except "nothing" (and its synonyms), denotes an existence, and is therefore a term. However, "nothing" is subject to the same logical operations as a term, hence we agree to regard it as such.

Def. 50a. A term is a word or phrase (or symbol) which denotes an existence; moreover, "nothing" (with its synonyms) is a term.

Def. 51. A proposition is a set of terms which denotes an alleged fact, or event. (See XXII, XXIII.)

Def. 52. A proposition is said to be true if it denotes a fact or event, false if it does not.

Def. 53. A statement is a proposition, or several propositions.

33. Contradictions.

Def. 54. A contradiction is a statement which is presumed to denote an inconsistency.

Def. 55. A non-contradiction is a statement which is presumed to denote a consistency.

A statement of the type "*A* is *B*" is a contradiction if *A* and *B* stand for terms which are presumed to denote different existences, and "is" denotes (and is presumed to denote) "is equivalent to". Thus "a cameleopard is a giraffe" is a contradiction to a person who knows what a giraffe is, but believes that a cameleopard is a leopard with a hump on its back. A statement of this type is a non-contradiction if *A* and *B* stand for terms which denote (and are presumed to denote) the same existence, and "is" denotes (and is presumed to denote) "is equivalent to".

If there were no uncertainty about the meaning of statements, every contradiction would denote an inconsistency, and every non-contradiction

would denote a consistency. But since different terms may denote the same existence, and since the same term may denote different existences, there are contradictions which do not denote inconsistencies, and non-contradictions which denote inconsistencies. Thus the statement "two is different from too", when heard, is usually a contradiction, because "two" and "too" are assumed to denote the same existence; but it would not denote an inconsistency unless it were assumed that the existences denoted by "two" and "too" are the same. Similarly, the statement "two is the same as too", when heard, would usually be a non-contradiction, but it would denote an inconsistency.

A statement of the type " A is not A " is a non-contradiction if "is not" denotes (and is presumed to denote) "is not equivalent to", and A stands for a term which denotes (and is presumed to denote) first one existence, and then a different existence. It is a contradiction if "is not" denotes (and is presumed to denote) "is not equivalent to" and if both A 's are presumed to denote the same existence; but it does not necessarily denote an inconsistency, because the presumption that both A 's denote the same existence may be false.

A type of statement which frequently arises in Logic, is " A is B and A is not B ", where both A 's—and both B 's—are presumed to denote the same existence, and each "is" is presumed to have the same meaning. Such a statement is a contradiction, but there are various cases in which it does not denote an inconsistency. *First*, the verb "is" may be used with, or without, reference to number, time and place; "the sun is eclipsed" and "the sun is not eclipsed" will be consistent (*i. e.* will denote a consistency) if the former "is" is unlimited, and the latter refers to "here and now". *Secondly*, a term may denote either a percept or a concept; "a printed word is internally connected" and "a printed word is internally separated" are consistent if connected denotes a concept and separated denotes a percept. *Thirdly*, there is a distinction in thought between the concept of a percept, and the concept of a concept; the concept of a printed word is internally separated if separated denotes the concept of a percept, but internally connected if connected denotes the concept of a concept.

It is necessary to restrict the choice of terms in such a way that it can easily be recognized whether a statement denotes a consistency, or an inconsistency.

34. Logical Restriction. Any proposition can be supposed to restrict the meaning of every term contained in it; because some meanings of any term would be inconsistent with the proposition. Thus "motion is change of position" can be supposed to restrict the meaning of each of the words "motion", . . . , "position". On the other hand, it can be supposed that the meaning of some of these words is determined in some other manner, and that the proposition merely restricts the meaning of the rest. If the meanings of "is", . . . , "position" are otherwise determined, the proposition merely restricts the meaning of "motion".

We shall say that an existence is *logically restricted* if the term which denotes it is involved in a proposition which restricts, or partially explains, the meaning of that term. Otherwise expressed, an existence is logically restricted if it is restricted, *i. e.* partially explained, through being involved in a fact or event.

Likewise, an existence is *logically unrestricted* if the term which denotes it is not involved in any proposition which restricts the meaning of that term. Or, an existence is logically unrestricted if it is not restricted through being involved in a fact or event.

35. Absolutes. We have explained number, time, space, persons, *etc.* by means of existence, consciousness, separation, distinction, succession, sensates, concepts and mental faculties. The latter are our fundamental existences; we have made no statement about them which can be used in logic, except that they can be distinguished from one another. The list of fundamental existences is incomplete, for we have only considered those which necessarily enter into logic.

If the fundamental existences should differ in different persons, no one would be aware of the fact; for no contradiction would arise in the exchange of ideas. Thus if an object which gives me the sensation of redness, gives another person the sensation which I would call yellow, but which he calls red, we will not be aware that our sensations are different. Again, if the fundamental ideas and memory should change alike in the same person, he or she would not be aware of any inconsistency.

We assume, however, that the fundamental existences do not vary; for otherwise there would be inconsistencies, though undetected, in thought and in the exchange of ideas. We express this assumption by stating that the fundamental existences are *absolutes*.

Def. 56. An *absolute* is an existence which is logically unrestricted except through being distinguished from all other existences; but it is assumed to be the same, whenever, wherever, and by whomsoever it is sensed or conceived.

With the progress of Science, the number of absolute sensates is continually being reduced. Geometry reduces sizes and forms to points and positions. Physics reduces sounds, and attempts to reduce nearly all other sensates, to motions of particles (or, of late, to singularities of space).

36. Implicates and Compounds.

Def. 57. We shall use the term *implicate* to denote an existence which (1) is distinguished from all other existences, (2) is logically restricted in some other manner, (3) is not a combination of absolutes, (4) is assumed to be the same at all times and places, and for all persons. *E. g.* thing, person.

(The following round-about method of expressing (2) may be more easily understood: The term which denotes an implicate is involved in one or more postulates, not of the type "*A* is different from *B*", which restrict the implicate.)

Def. 58. A *compound* is wholly composed of absolutes or implicates, or both. *E. g.* a white person.

(The term which denotes a compound, can be defined.)

When facts or events are discovered which restrict absolutes, the latter become implicates, or compounds. Thus we have regarded certain colors as absolutes; but in Physical Optics, they are compounds.

37. Definitions.

XLII. We can denote each absolute, and each implicate, by a distinctive term. (See Note, below.)

Def. 59. An **undefined term** is a term which denotes an absolute or an implicate.

If an undefined term should be ambiguous (*e. g.* in the manner described in §33), the ambiguity can be removed by an adjective or phrase (or pre-determined convention). Thus "separation" may denote either a percept or a concept; but "perceptual separation" denotes a single existence. Then, just as compounds are formed from absolutes and implicates, compound terms can be formed from undefined terms. Thus it is possible to denote each existence by one and only one term.

The description of a compound by means of undefined terms is usually very inconvenient, hence this description is usually replaced by a word or convenient phrase.

Def. 60a. Let *B* denote a description of a compound by means of undefined terms, and let *A* denote the word or phrase which may replace *B*; then "*A* is equivalent to *B*" is a **definition of *A***.

Thus we see that it is often desirable to have more than one means of denoting the same existence. But employing the same term to denote different existences leads to confusion.

XLIII. Every term (except "nothing" and its synonyms) should denote one and only one existence.

An example of **Def. 60a** is "A peninsula is a piece of land almost surrounded by water". This type of definition applies only to compounds.

An implicate is, or can be regarded as, a unit. It may be abstract, *e. g.* a person, or concrete, *e. g.* John Smith. An abstract implicate possesses no property or condition except those which are implied by the postulates which restrict it. A concrete implicate usually possesses further properties and conditions. Thus a *concrete persistent percept* possesses known properties, and new properties can always be discovered in it (see XXI). Hence such a percept is a concrete implicate, but the postulates which restrict it have not all been formulated.

Def. 60b. The postulates which restrict an abstract implicate may be called a *definition of the implicate*.

Def. 60c. The concrete implicates which satisfy these postulates (of **Def. 60b**) constitute a class. These postulates may likewise be called a *definition of the class*.

Def. 60d. If we can find several classes *A, B, C, . . .* such that a concrete implicate *c* is the only unit which is contained in all of them, "*c* is contained in *A, B, C, . . .*" is a *definition of c*.

Cor. If we can find several abstract implicates *A', B', C', . . .* such that a concrete implicate *c* is the only unit which is an example of each of them, "*c* is an example of *A', B', C', . . .*" is a *definition of c*.

Thus a particular book in a library is defined in the catalogue by means of the name printed on each book of the edition, the author's name, the place and year of publication, *etc.*

Note. Concrete implicates are so numerous that it is impossible to denote each of them by a distinctive term, unless a definition is so regarded. This occasions no difficulty in the definition of a compound, for a compound is abstract (since it has no unknown property or condition) and is therefore formed from absolutes and *abstract* implicates.

A concrete implicate can usually be defined in various ways. To some extent, the same is true of abstract implicates and compounds. Two defini-

tions are said to be *equivalent* if they define the same existence. (See Def. 64.)

If all terms are chosen in the way described in this article, and all definitions are the same (or equivalent) for everyone, every contradiction will denote an inconsistency, and every non-contradiction will denote a consistency. There may still be inconsistencies, for our judgment in regard to which existences are absolutes *etc.* may be at fault.

38. Demonstrations. In §5, we stated that a demonstration is a syllogism, or a succession of syllogisms. It is true that a demonstration can always be presented in this form, but essentially, it is a process of selecting and restating propositions which are asserted to be true, so as to obtain an assertion of the truth of a desired proposition. It is not necessarily expressed in syllogisms.

The selection, or sequence, of propositions is directed by the principle that there must be no inconsistencies. We assert that the following propositions are true, and that any statement which is inconsistent with them is false; they assist us in selecting propositions, and in restating them.

XLIV. A percept must possess a property, or *not* possess it.

XLV. A percept cannot possess a property, and also *not* possess it.

XLVI. Either there is, or there is not, a percept which possesses a given property.

XLVII. It is impossible that there should be, and also *not* be, a percept which possesses a given property.

XLVIII. At a given instant, a persistent percept must either possess a condition, or *not* possess it.

XLIX. At a given instant, a persistent percept cannot possess a condition, and also *not* possess it.

L. Either there is, or there is not, a persistent percept which possesses a given condition at a given instant.

LI. It is impossible that there should be, and also *not* be, a persistent percept which possesses a given condition at a given instant.

In place of percept, property, condition in the above postulates, we may write concept, conceived property, conceived condition. If a condition is not natural (Def. 48), XLVIII, . . . LI must be restricted either to the persons who can perceive the condition, or to those who cannot perceive it.

LII. If a sensate is possessed during any time and in any place, it is possessed during any part of that time, and in any part of that place.

LIII. If a sensate is sensed, and distinguished into other sensates, these sensates are sensed.

E. g. if a percept possesses a property a , and if a is resolved into a' , a'' , then it possesses the properties a' , a'' .

LIV. If several sensates are sensed, and if they are "indistinguished" into a single sensate, this sensate is sensed.

The *restating* of propositions is effected by means of the following principles:

LV. Any term can be replaced by a term which denotes the same existence.

LVI. Any proposition can be replaced by a proposition which denotes the same fact or event.

As an example of LV, suppose that a percept A does not possess the property a . Then we say that A "does not possess the property a ", or that it "possesses the property not- a ". The terms in quotation marks have the same meaning, and either of them can replace the other. Similarly, "possesses the property a " can replace or be replaced by "does not possess the property not- a ".

39. Postulates. Since every demonstration must begin with a proposition, there must be undemonstrated propositions.

Def. 61. A **postulate** is an unproved proposition which is assumed to be true.

The truth of some postulates, *e. g.* those in Sects. II & III, and in §38, is asserted. Other postulates, *e. g.* "Gravitation is propagated instantaneously", are merely assumed to be true; to assume that such a postulate is false is not inconsistent with our logical background.

An exact science (§7) contains propositions which are demonstrated from logical postulates, and Natural Laws. The logical postulates are the same for all sciences, and are asserted to be true. The Natural Laws are peculiar to the science, and are merely assumed to be true; they form a "set of postulates".

Def. 62. A **consistent set of postulates** is a set of postulates which does not lead to an inconsistency.

LVII. A set of postulates must be consistent.

If the set is not consistent, the postulates cannot all be true; some of them must be altered, until the inconsistencies are removed.

The postulates of a set should also be *independent*; *i. e.* no one of them should be demonstrable from the rest. Moreover, the individual postulates should not be *redundant*; *i. e.* no part of any postulate should be demonstrable from the rest of the set.

Def. 63. A **theory** is a set of set of postulates, together with a set of propositions demonstrated from them.

Def. 64. Two propositions are said to be **equivalent** if either of them can be demonstrated from the other.

Def. 65. Two sets of postulates are said to be **equivalent** if each set can be demonstrated from the other.

LVIII. If we have a set of true postulates, and if we can prove that this set and another proposition lead to an inconsistency, the proposition is false.

40. Various Definitions.

Def. 66. A **theorem** is a proposition (usually an implication) which is to be demonstrated.

Def. 67. A **corollary** of a proposition is another proposition which can easily be demonstrated from it.

Def. 68. A **lemma** is a proposition which is required for the demonstration of some other proposition.

Def. 69. A **problem** is a proposition in which either the hypothesis or the conclusion is to be discovered; when a problem is solved, it can be stated as a theorem.

Def. 70. **Sufficient conditions**, or conditions sufficient for the truth of a proposition, are propositions from which that proposition can be demonstrated.

Def. 71. Necessary conditions, or conditions necessary for the truth of a proposition, are propositions which must all be true, if the given proposition is to be true; *i. e.* if we assume that any one of these propositions is false, we can prove that the given proposition is false.

***41. Explanation of an Inconsistency.** Let us consider the proposition (A) "Hephaestus the Cretan said 'All Cretans are liars' ". This proposition does not introduce an inconsistency unless we associate with it the implied proposition (B) "Whatever a Cretan says about Cretans is true". The inconsistency is obtained in the following way.

Because Hephaestus is a Cretan (C) "All Cretans are liars" is true; for it is a statement made by a Cretan, about Cretans. But since Hephaestus is a Cretan, (C) includes the statement "Hephaestus is a liar"; and since Hephaestus is a liar, his statement (C) is false. Hence (C) is both true and false, which is inconsistent with the principle that a statement cannot be true, and also false. (See §47.)

We shall assume, as seems to be intended, that (C) is a categorical proposition. Then "are" is unlimited in time, and (C) is always true, or always false (§47). Now the term "liars" may be interpreted in either of two ways. A liar may be a person who (1) lies whenever he makes a statement, or (2) sometimes lies and sometimes does not lie when he makes a statement.

If (C) is false then, since this is a statement of a Cretan about Cretans, (B) is false.

If (C) is true, there is no inconsistency unless Hephaestus told a lie when he said (C); in this case, a Cretan made an untrue statement about Cretans, that is (B) is false.

Either there is no inconsistency, or (A) contradicts (B). In the latter case, we have inconsistent postulates; not a contradiction of a logical principle.

V. CATEGORICAL LOGIC

42. Categories.

Def. 72. A **category** is a number of units, each of which possesses a stated property, or stated properties.

A category is a *class*. In the rest of this chapter we always write "class" with the meaning of category.

The distinction between "a property" and "properties" is not essential in the definition of a class; a property may be distinguished into several properties, or several properties may be merged into a property. The definition of a sub-class, however, requires several properties.

Def. 73. A unit which possesses the stated property, or stated properties, is **contained in the class**.

Def. 74. A unit which does not possess the stated property, or each of the stated properties, is **not contained in the class**.

Theorem I. *Every unit is either contained in a class, or not contained in the class*

For every unit must either possess the stated property, or *not* possess it (XIV). Hence, by Defs. 73 & 74, every unit is either contained in the class or not contained in the class.

Theorem II. *A unit cannot be contained in a class, and also not contained in the class.*

For a unit cannot possess a property, and also not possess it (XLV).

Def. 75. The **complement** of a class is a number of units, each of which does not possess the stated property, or each of the stated properties.

Theorem III. *The complement of a class is a class.*

For each unit of the complement possesses the following property: "The stated properties of the units of the original class cannot, each of them, be perceived (or conceived) in it". Thus the complement is a number of units, each of which possesses a stated property; that is, it is a class (Def 72).

Cor. 1. *Every unit is contained in a class, or in the complementary class.*

For every unit either possesses the stated property of the class, or it does not possess it (XLIV). That is, it possesses either the stated property of the class, or that of the complement. That is, (Def. 73), it is contained in the class, or in its complement.

Cor. 2. *A unit cannot be contained in a class, and also in the complementary class.*

For a unit cannot possess the stated property of the class, and also not possess it (XLV). That is, it cannot possess the stated property of the class, and also that of the complement. That is, it cannot be contained in the class, and also in its complement (Def. 73).

Def. 76. Two classes are said to be **identical** if their stated properties are the same.

A unit class is identical with the unit which it contains if, and only if, the stated properties of the class are identical with the properties of the unit; hence (XXI) a unit class is not identical with a concrete persistent unit.

Theorem IV. *The complement of the complement is a class which is identical with the original class.*

For it is a number of units each of which possesses the stated properties of the original class.

Def. 77. Two classes are said to be **equivalent** if they contain the same units.

Two equivalent classes are not necessarily identical, since their stated properties may be different.

In Mathematical Logic, classes are denoted by letters. If a class is denoted by A , its complement is denoted by not- A . By Theorem IV, the complement of not- A is A .

The symbol " \equiv " is used to denote either identity or equivalence. The symbol " $($ " is used to denote "is contained in", and " $)$ " is used for "contains".

43. Categorical Propositions.

Def. 78. A **categorical proposition** is a statement that some or all units of a class are contained in another class.

Def. 79. A categorical proposition is **true** if each of the units indicated possesses the stated properties of the second class.

If the properties of the second class are properties of the first class, the proposition is said to be *true in itself*; e. g. "All equilateral triangles

are isosceles". The proposition "All horses are animals" is true in itself if, and only if, the definition of "horses" includes every property stated in the definition of "animals". Thus it may not be evident whether a proposition is true in itself, or not; for a definition may be variously stated, and need only include sufficient properties to distinguish whatever is defined from all other existences.

Of categorical propositions which are not true in themselves, some are asserted to be true, *e. g.* certain logical postulates (see §39); and others are merely assumed to be true, *e. g.* "Every even positive integer is the sum of two primes". When possible, the latter are *demonstrated*.

It may be said that Def. 78 does not include such a proposition as "Honesty is a policy". But "Honesty" is a monad (§14), since it is distinguished from other existences; it can therefore be regarded as a unit, and a unit is a kind of class. Likewise a policy must have some properties by which it is recognized as a policy, hence policies can be regarded as a class. Thus "honesty" is a unit which is contained in the unit class "honesty", and also in the class "policies".

In scholastic logic, it is customary to represent units by letters, and to call classes, or parts of classes, *terms*; the terms of the proposition "All horses are animals" are "All horses" and "animals". Terms are distinguished into the types *universal affirmative*, *universal negative*, *particular affirmative*, *particular negative*; these are respectively represented by "All A's", "All not-A's", "Some A's", "Some not-A's". Propositions are distinguished into like-named types, each type including two varieties; *e. g.* the two varieties of universal affirmative propositions are represented by "All A's are B's", and "All A's are not-B's".

Def. 80. The **converse** of a proposition is obtained by interchanging its classes. Thus the converse of "Some A's are B's" is "Some B's are A's".

Def. 81. The **obverse** of a proposition is obtained by replacing each class by its complement. Thus the obverse of "Some A's are B's" is "Some not-A's are not-B's".

Def. 82. The **contra-positive** (or *negative converse*) of a proposition is obtained by replacing each class by the complement of the other. Thus the contra-positive of "Some A's are B's" is "Some not-B's are not-A's".

The following theorems can easily be verified:

Theorem V. *Any proposition is the same as the converse of its converse. Likewise it is the same as the obverse of its obverse; and it is the same as the contra-positive of its contra-positive.*

Theorem VI. *In any proposition, the contra-positive of the converse is the obverse. Likewise the contra-positive of the obverse is the converse.*

Def. 83. The **contrary** of a categorical proposition is obtained by replacing "all" by "some" (or "some" by "all") in the first term, and by replacing the second class by its complement.

Thus the contrary of "All A's are B's" is "Some A's are not-B's".

Def. 84. A categorical proposition is said to be **false**, if its contrary is true.

Thus a universal categorical proposition is false if some (*i. e.* one or more) units of the first class are not contained in the second class. A particular categorical proposition is false if every unit of the first class is not contained in the second class.

Theorem VII. *If a categorical proposition is true, its contrary is false.*

Proof. Every categorical proposition is identical with the contrary of its contrary. Hence if a categorical proposition P is true, the contrary of the contrary of P is true; i. e. (Def. 84) the contrary of P is false.

44. Sub-classes.

Def. 85. Let A represent the class whose units possess the property (or properties) a ; B , the class whose units possess the property b ; and AB the class whose units possess the properties a and b . Then AB is said to be a **sub-class** of A , and also of B .

Cor. 1. Every unit of AB is contained in A , and also in B .

Cor. 2. If b is the same as a , AB is identical with A , and with B ; thus every class is a sub-class of itself.

Here an inconsistency would arise if it were assumed that AB is different from A . But since A and AB are defined, the inconsistency would be evident.

Def. 86. If A is a sub-class of B , B is said to be a **super-class** of A .

The symbol “ (” is used to denote *is a sub-class of*, and “) ” is used for *is a super-class of*.

Def. 87. Let A denote the class whose units possess the property a , and let not- B denote the class whose units possess the property not- b ; i. e. (see LV) do not possess the property b ; also let A .not- B denote the class whose units possess the properties a and not- b . Then A .not- B is a sub-class of A (Def. 85); it is called the **complementary sub-class** of AB with respect to A .

Ex. 1. Every unit of A is contained in AB , or in A .not- B .

Ex. 2. A unit of A cannot be contained in AB , and also in A .not- B .

Ex. 3. If A is a sub-class of B , and C is another class, AC is a sub-class of BC .

The application of some of the theorems of this chapter is restricted by the condition that the classes referred to, must exist. It is usually considered desirable to extend the definition of a class, so that these theorems can always be applied.

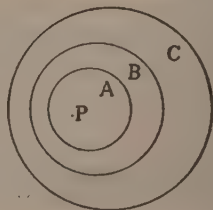
If a unit which possesses certain properties does not exist, we can represent the fact by the statement: “There are *no* (or *zero*) units which possess the stated properties”. Now we can regard “zero” as a number, and “Zero units which possess certain stated properties” as a class.

Def. 88. A zero class is *zero* (or *no*) units, with a stated property, or stated properties.

LIX. All zero classes are equivalent.

45. Theorem VIII. *If A is a sub-class of B and B is a sub-class of C , then A is a sub-class of C . (See §47.)*

The demonstration of this theorem is made somewhat clearer by means of a diagram. Let the class C be represented by the interior of a circle C ; the class B , by the interior of a circle B lying within C ; and the class A , by the interior of a circle A lying within B . Also let any unit of A be represented by a point P lying within the circle A . The theorem itself is represented by the statement that every point P within A , lies in the interior of C .



Proof. Every unit of the class C possesses some property (or properties) c . Because B is a sub-class of C , every unit of B possesses the property c , and also some property b (Def. 85). Because A is a sub-class of B , every unit of A possesses the properties b and c , and also some property a . Thus every unit of A possesses the property c , and is therefore contained in C .

This theorem may be stated in symbols, thus: "If $A (B$ and $B (C$, then $A (C$.

Cor. 1. If $A) B$ and $B) C$, then $A) C$.

Cor. 2. If all A 's are B 's, and all B 's are C 's, then all A 's are C 's.

Note. The equivalence of propositions, like that of classes, is denoted by " \equiv "; thus we write $A (B \equiv B) A$.

Cor. 3. If $A \equiv B$ and $B \equiv C$, then $A \equiv C$.

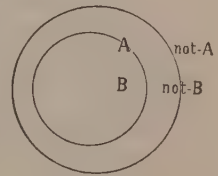
For since $A \equiv B$, $A (B$ and $A) B$ (Def. 77). Similarly $B (C$ and $B) C$. Since $A (B$ and $B (C$, therefore $A (C$ (Theorem VIII). Similarly $A) C$ (Cor. 1). Thus $A (C$ and $A) C$; therefore $A \equiv C$ (Def. 77).

The type of categorical syllogism which is most frequently used in mathematics, is that given in *Cor. 2*; this is Theorem VIII, stated in another way. The various types of true categorical syllogisms which are listed in works on logic, can be demonstrated similarly.

The major premise of a syllogism is sometimes called a *maxim*.

46. Theorem IX. If $A) B$, then $\text{not-}A) \text{not-}B$.

This relation is represented in the figure. The classes A and $\text{not-}A$ are represented by the interior and exterior, respectively, of the larger circle; and the classes B and $\text{not-}B$, by the interior and exterior of the smaller circle. We observe that if the interior of the larger circle contains the interior of the smaller, the exterior of the smaller circle will contain the exterior of the larger.



Proof. If $A) B$, then A contains every unit of B ; hence, by Theorem III, *Cor. 2*, $\text{not-}A$ does not contain any unit of B ; i. e. every unit of $\text{not-}A$ is not a unit of B . Therefore, by Theorem III *Cor. 1*, every unit of $\text{not-}A$ is a unit of $\text{not-}B$; that is, $\text{not-}B$ contains every unit of $\text{not-}A$; that is, $\text{not-}B) \text{not-}A$.

Cor. 1. If $\text{not-}B) \text{not-}A$, then $A) B$.

Cor. 2. $A) B \equiv \text{not-}B) \text{not-}A$.

Cor. 3. Every universal categorical proposition is equivalent to its contra-positive.

Cor. 4. $(A \equiv B) \equiv (\text{not-}A \equiv \text{not-}B)$.

Cor. 5. It follows from §37, that every definition can be expressed in the form $A \equiv B$. Hence every definition is equivalent to its converse, obverse and contra-positive.

47. Demonstrations. Theorems VIII and IX denote facts about classes; they are statements that if some categorical propositions are true, then certain other categorical propositions are true. They could equally well be expressed in the form "Because . . . , therefore . . . "; they would then be assertions that some categorical propositions are true, and that consequently certain other propositions (whose truth was doubt-

ful) are true. This is one method of demonstrating conditional propositions.

Another method, which is based upon Theorem XIV, requires the following propositions:

Theorem X. *A universal categorical proposition must be true or false.*

Theorem XI. *A universal categorical proposition cannot be true and false.*

Proof. Let the proposition be $A (B$; and let the stated properties of the classes A and B be a, b , respectively. Then

$A (B$ is true means every unit of A is in B (Defs. 79, 73); and therefore no unit of A is in not- B (Theorem III, Cor. 2); hence no unit possesses a and also not- b (Def. 72); which means that no unit possesses a .not- b . (For a and not- b , taken together, form a property; and this property is denoted by a .not- b .)

Similarly, $A (B$ is false means some unit possesses a .not- b .

Now there is, or there is not, a unit which possesses a .not- b (XLVI), hence $A (B$ is true or false.

And there cannot be a unit which possesses, and also does not possess, a .not- b (XLVII), hence $A (B$ cannot be true and false.

Cor. A particular categorical proposition is either true or false, and it cannot be both true and false.

Let the proposition be "Some A 's are B 's" (1)

The contrary of (1) is "All A 's are not- B 's" (2).

Since (2) is a universal categorical proposition, it is either true or false, and it is not both true and false.

Hence (Def. 84 and Theorem VII), (1) is false or true, and is not both false and true.

Def. 89. A unit categorical proposition is a statement that the unit of a unit class possesses a stated property, or stated properties.

The contrary of the unit proposition "The unit A is contained in B " is "The unit A is contained in not- B ". Each of these is a *universal* proposition, since "The unit A " includes every unit of the class A .

VI. HYPOTHETICAL LOGIC

48. Conditional Classes.

Def. 90. A conditional class is a number of units, each of which possesses stated properties or conditions.

There are many special cases, which cannot be treated in detail. We shall state these cases, and indicate the necessary modification of the standard treatment. If there are stated conditions, some of these may be personal, not natural. In such cases the persons who perceive the conditions must be indicated. Again, conditions may depend upon time, or upon both time and place. If they depend upon time only, the references to place in the standard treatment must be omitted. In some cases, the class itself may be considered only throughout a limited time, or time and place. In the standard case, a class is considered throughout every time and place; to adapt it to other cases, the time, or time and place, must be indicated. Units which possess the stated properties or conditions may be created or destroyed. This fact does not affect the truth of the theorems in this chapter; the theorems contain the necessary assertions as to existence.

If only *properties* are stated, the conditional class is a category. If *conditions* are stated, it will be impossible to tell whether a unit is contained in the class unless a time and place are mentioned, throughout which the unit possesses, or does not possess, the conditions. This is always possible, because at a given instant a unit either possesses or does not possess a stated condition (XLVIII). If the condition depends upon the place, the unit is an object and its position at a given instant is determined (XXXII).

LX. A conditional class exists at every time and place.

The category "All men", with the restriction "This class exists only on the earth, during the present geological period" is not a conditional class. But "All men who live upon the earth during the present geological period", with no restriction, is a conditional class which exists, but contains no units, at times and places not included in the criterion.

We obtain a sufficient account of conditional classes from §42, by replacing "category" (or "class") by "conditional class at a given time and place."

49. Conditional Propositions.

Def. 91. A conditional proposition is a statement that some or all units of a conditional class are contained in another conditional class.

Thus "The stars are shining" is a statement that the units of the conditional class "The stars" are now contained in the class of "shining" units, hence it is a conditional proposition.

If the conditional classes are categories, the proposition is a categorical proposition.

A conditional proposition which is not categorical refers to a given time and place, or to given times and places; without such a reference, it has no definite meaning.

Def. 92. A conditional proposition is **true at a given time and place** if each of the units indicated is contained in the conditional class at that time and place.

The account of categorical propositions in Sect. V applies to conditional propositions at a given time and place; hence we give only an abbreviated treatment of the latter.

Def. 93. The **contrary** of a conditional proposition is obtained by replacing "all" by "some" (or "some" by "all") in the first term, and by replacing the conditional class by its complement.

Thus the contrary of "All men are happy" is "Some men are not happy".

Def. 94. A conditional proposition is said to be **false at a given time and place**, if its contrary is true at that time and place.

It follows from §47, that:

Theorem XII. *At a given time and place, a conditional proposition must be true or false.*

Theorem XIII. *At a given time and place, a conditional proposition cannot be both true and false.*

Def. 95. A **unit conditional proposition** is a statement that the unit of a unit conditional class is contained in a given conditional class.

Def. 96. A **class of conditional propositions** is a number of unit conditional propositions, each of which is recognized in some manner. (*E. g.*

the units of each proposition may be contained in a given category; examples occur in implications).

50. Immediate Implications. First let us consider the statement

(A) When the actors are performing well, the spectators are pleased. Since no place is mentioned, we infer that the statement refers to any place. It informs us that

(B) The actors perform well at certain times, and that

(C) During any good performance, the spectators are pleased.

Let us now consider the statement

(D) If the actors are performing well, the spectators are pleased.

This statement informs us that at times when the actors perform well (which times may, or may not, exist), the spectators are pleased; it has precisely the same meaning as (C). Thus (B) and (D), taken together, have the same meaning as (A).

Now (D) is an immediate implication. It can be expressed in the forms

(E) "The actors are performing well" implies "the spectators are pleased",

(F) "The spectators are pleased" is implied by "the actors are performing well".

Def. 97. An immediate implication is a statement that times and places at which one conditional proposition is true, are times and places at which some other conditional proposition is true.

Thus an immediate implication is simply a statement that a conditional proposition is true throughout various times and places. The circumstance that these times and places are determined by the truth of some other conditional proposition is of secondary importance.

By "times and places" we mean one or more intervals of time, at each instant of which one or more places are considered. Thus the times and places at which there are floods are the times of the floods, together with the areas which are flooded at each instant of these times.

In hypothetical logic, conditional propositions are usually denoted by letters. Thus any immediate implication can be represented by "If X is true, then Y is true" The symbol " \cdot " is used to represent *implies*, and " \cdot " is used for *is implied by*. Thus any immediate implication can be represented by $X \cdot Y$, or by $Y \cdot X$.

Def. 98. The immediate implication $X \cdot Y$ is true, if Y is true at the times and places at which X is true.

" $X \cdot Y$ " is understood to mean " $X \cdot Y$ is true", unless the contrary is stated.

If there is no time and place at which X is true, then $X \cdot Y$.

Def. 99. The immediate implication $X \cdot Y$ is false, if the contrary of Y is true at some time and place at which X is true.

Def. 100. An example in which the contrary of Y is true at a time and place at which X is true, is called an example to the contrary of $X \cdot Y$.

In the implication "If X is true, Y is true", it may happen that " X is true" includes " Y is true"; e. g. "If a triangle is equilateral, it is isosceles". Such an implication may be called *true in itself*.

Def. 101. Two implications are said to be **equivalent**, when either of them can be demonstrated from the other.

Thus $X \cdot Y$ is equivalent to $Y \cdot X$; this is denoted by $X \cdot Y \equiv Y \cdot X$.

The processes of conversion, obversion and contra-position are the same for implications as for categorical propositions, except that instead of replacing a class by its complement, we replace a conditional proposition by its contrary. Theorems V & VI also apply to implications.

51. Various Theorems on Immediate Implications.

Theorem XIV. If $X \cdot Y$ and X is a true categorical proposition, then Y is a true categorical proposition.

Proof. " X is a true categorical proposition" means " X is true at all times and places". " X is true at all times and places" and " $X \cdot Y$ ", together, have the same meaning as " Y is true at all times and places"; i. e. they have the same meaning as " Y is a true categorical proposition".

Theorem XV. If $X \cdot Y$ and $Y \cdot Z$, then $X \cdot Z$.

Proof. Let T denote the times, with the associated places, at which X is true. Then " $X \cdot Y$ " has the same meaning as " Y is true throughout T ". Also " Y is true throughout T " and " $Y \cdot Z$ " together assert that Z is true throughout T (see LII); i. e. $X \cdot Z$.

Cor. 1. If $X \cdot Y$ and $Y \cdot Z$, and if X is a true categorical proposition, then Z is a true categorical proposition.

Cor. 2. If there are times and places at which X is true and Y is true, and if $Y \cdot Z$; then there are times and places at which X is true and Z is true.

We now require the two following postulates:

LXI. The times and places T throughout which any conditional proposition is considered, can be separated into times and places T_i ($i=1, 2, 3, \dots$) such that the proposition is true throughout T_i , or false throughout T_i .

LXII. If a conditional proposition is true throughout each of the times and places T_i ($i=1, 2, 3, \dots$) into which T is separated, it is true throughout T .

Theorem XVI. An immediate implication must be true or false, and it cannot be both true and false.

Let the implication be "Every unit of the conditional class A possesses the property or condition b throughout the times and places T "; and let Y denote the conditional proposition "Every unit of the conditional class A possesses the property or condition b ".

Proof. T can be separated into times and places T_i throughout which b is either possessed or not possessed (LXI). Throughout each T_i , Y is either true or false, and is not both true and false (Theorems XII & XIII). If Y is false in any T_i , the implication is not true (i. e. does not satisfy Def. 98), and is false (i. e. satisfies Def. 99). If Y is true in every T_i , it is true in T (LXII), and the implication is true, and is not false.

Theorem XVII. If $X \cdot Y$, then the contrary of Y is the contrary of X .

This theorem informs us that the assertion $X \cdot Y$ is true (which assertion may or may not be made) necessarily leads to the assertion that the contrary of Y is the contrary of X is true.

Thus from the assertion that (1) if X is true, then Y is true, we are to prove that (2) if the contrary of Y is true, then the contrary of X is true.

Proof. Suppose that the implication (2) is false; *i. e.* (3) there is some time and place at which the contrary of Y is true, and X is true. It follows from (3) and (1) that there is some time and place at which the contrary of Y is true, and Y is true, (Theorem XV, Cor. 2). This is inconsistent with Theorem XIII. Hence the supposition (3) is false (LVIII). Hence (2) is true (Theorem XVI).

Illustration. "If the actors are performing well, the spectators are pleased; hence if some of the spectators are not pleased, some of the actors are not performing well".

Cor. 1. The case in which X and Y are unit conditional propositions has special interest. From the implication "If the unit A possesses the property or condition b , then the unit C possesses the property or condition d " we can infer that "If C possesses not- d , then A possesses not- b ". *E. g.* "If a given plane rectilinear figure has 10 sides, the sum of its angles is 16 right angles"; hence "If the sum of the angles of the figure is not 16 right angles, it does not have 10 sides".

Cor. 2. If the contrary of $Y \cdot \cdot$ the contrary of X , then $X \cdot \cdot$ Y .

Cor. 3. $X \cdot \cdot$ $Y \equiv$ the contrary of $Y \cdot \cdot$ the contrary of X .

52. Demonstrations. Theorems XIV, XV, XVII are statements that if certain implications are true, then certain other implications (or categorical propositions) are true. The purpose of a demonstration is usually to arrive at the assertion that some implication or categorical proposition is true. Hence these theorems are commonly used in the form "Because . . . , therefore . . .".

The advantage of demonstrating categorical propositions by means of implications (other than Theorems VIII & IX) is chiefly due to the fact that there are unproved implications whose truth is asserted. Thus in arithmetic we have "If unity is repeatedly subtracted from any positive integer, it will ultimately be reduced to zero". In elementary text-books, they are usually disguised. We can make use of these asserted implications by introducing them into Theorems XIV, XV & XVII.

A *synthetic* demonstration is one which proceeds from the hypotheses to the conclusion (§4); it therefore involves syllogisms of the form "because $X \cdot \cdot$ Y and $Y \cdot \cdot$ Z , therefore $X \cdot \cdot$ Z ".

An *analytical* demonstration is one which proceeds from the conclusion to the hypotheses; hence it involves syllogisms of the form "because $Z \cdot \cdot$ Y and $Y \cdot \cdot$ X , therefore $Z \cdot \cdot$ X ". This method is frequently used, in cases where the conclusion is known, as a means of discovering synthetic demonstrations. In mathematics, however, there seems to be a tendency to employ "analytic" as a synonym of "admirable".

The method of demonstration known as *reductio ad absurdum* consists in taking the contrary of a proposition which it is desired to prove, as a postulate; and constructing a demonstration—in which all the hypotheses excepting this postulate are asserted to be true—which ends in a contradiction. We conclude (LVIII) that this postulate is false; hence the proposition must be true. The contradiction may be either a proof that the added postulate is false, or a proof that one of the other hypotheses is false. The proof of Theorem XVII is an example of this method.

53. Implications in General. It is difficult to give a general definition of an implication. Immediate implications are the simplest, and they are those which are ordinarily employed. There is another variety in which the conclusion is true, *not* throughout times and places T in which the hypothesis is true, but throughout times and places U which becomes known when T is known; these may be called *deferred* implications. In a third variety, which may be called *coupled* implications, there is a one to one correspondence between the units of the conditional classes involved in the hypothesis and the conclusion. Immediate implications frequently, and deferred implications usually, are coupled. We shall not discuss deferred implications which are not coupled.

54. Coupled Implications. An example of an implication which is of the first and third varieties is

(1) "If teams are winning, their supporters are happy".

Hence, each team has its group of supporters. Regarding (1) as of the first variety, we obtain by contra-position

"If some supporters are not happy, some teams are not winning".

But because it is of the third variety, we can also infer

(2) "If all groups of supporters are not happy, all teams are not winning".

In an implication which is not coupled, such a universal inference cannot be drawn; *e. g.* from

"If the actors are performing well, the spectators are pleased" we *cannot* infer

"If all of the spectators are not pleased, all of the actors are not performing well".

It is easy to explain why the inference (2) can be made. In a coupled implication we have two classes of unit conditional propositions (Defs. 95 & 96). Thus

(3) "If this team is winning, its supporters are happy" is an implication in which each conditional proposition is a unit proposition, since a team and its group of supporters are each class-units; and (1) is an implication in which "teams are winning", "supporters are happy", are classes of unit conditional propositions.

We can, in fact, call (3) a unit implication, and (1) a class of implications.

From (3) we infer that

"If the supporters are not happy, the team is not winning". This is a unit implication, and the class of unit implications in which it is contained is (2).

It will be observed that (1) is a more effective implication than (3), because the more general the implication, the more probable it becomes that the conclusion depends upon the stated hypothesis, and not upon some extraneous circumstances. An implication in which there is only one instance of the hypothesis does not indicate any such dependence.

In the coupled implication "If A has the condition b at times and places S , then C has the condition d at times and places T ", we can obtain a class of conditional propositions in the hypothesis, (I) by varying A , requiring that the A 's shall be contained in a certain category, or (II) by varying b , requiring that the b 's shall be referred to the same scale (§19),

or (III) by varying both A and b . With each unit proposition of the hypothesis is coupled a unit proposition of the conclusion; and with each time and place at which the former is true is coupled a time and place at which the latter is true. The following are examples of coupled implications which involve classes of conditional propositions:

"If employees work in a factory during the week, their wages are paid in the office on the following Saturday".

"As a man grows older, he grows wiser".

Def. 102. Let X_i denote a unit conditional proposition of the class X , and let S_{ij} denote a space-time interval, *i. e.* an interval of time and a place, such that throughout this interval X_i is either true or false; also let Y_i denote a unit conditional proposition of the class Y , and T_{ij} a space-time interval throughout which Y_i is either true or false. Then the coupled implication $X \cdot Y$ is defined by the following postulates:

(I) Each proposition X_i is coupled with a proposition Y_i .

(II) Each interval S_{ij} is coupled with an interval T_{ij} .

(III) It is stated that if X_i is true in the interval S_{ij} , Y_i is true in the interval T_{ij} .

There is no criterion for the class X , except that its units must be conditional propositions. So far as the definition is concerned, the X_i may be chosen arbitrarily. The X_i determine the Y_i , and the totality of these is Y .

55. Coupled Implications (continued).

Def. 103. A coupled implication is said to be **true**, if the statement in (III) is true.

Def. 104. A coupled implication is said to be **false**, if any X_i is true in any interval S_{ij} , and the corresponding Y_i is false in the corresponding interval T_{ij} .

Def. 105. A proposition X_i , considered in an interval S_{ij} , is called an **instance** (or *case*) of X .

Def. 106. An example in which a case of X is true and the corresponding case of Y is false, is called an **example to the contrary** of the implication.

Suppose that X_i denotes "The unit A_i has the condition b_i ". The contrary of X_i is "The unit A_i has the condition not- b_i "; this can conveniently be denoted by not- X_i . Thus the contrary of X , *i. e.* the class of propositions not- X_i can be denoted by not- X . Hence the obverse of $X \cdot Y$ will be denoted by not- $X \cdot$ not- Y , and its contra-positive will be denoted by not- $Y \cdot$ not- X . Theorems V, VI apply to coupled implications.

Theorem XVIII. If $X \cdot Y$, not- X_i is coupled with not- Y_i .

Proof. X_i is the contrary of not- X_i , and not- X_i is the contrary of X_i , hence X_i and not- X_i are coupled (Def. 22). Similarly Y_i and not- Y_i are coupled. Also X_i and Y_i are coupled, by hypothesis; hence not- X_i and not- Y_i are coupled (XIX).

Cor. If not- X_i is false throughout S_{ij} , not- Y_i is false throughout T_{ij} . Also if not- X_i is true throughout S_{ij} , then not- Y_i is either true or false throughout T_{ij} .

To prove Theorem XVa we require the two following postulates:

LXIII. Suppose that $X \cdot Y$ (or $Y \cdot X$), and that X_i is considered during an interval or intervals S_i in which X_i is sometimes true and some-

times false. Then S_i can be separated into intervals S_{ij} ($j = 1, 2, 3, \dots$) such that (1) X_i is either true or false throughout each S_{ij} , and (2) each S_{ij} is coupled with an interval T_{ij} throughout which Y_i is either true or false. The intervals T_{ij} constitute an interval or intervals T_i , in which Y_i is considered, and T_i corresponds to S_i .

LXIV. Suppose that $X \cdot Y$, and that X_i is considered during intervals S_i, S'_i , to which correspond intervals T_i, T'_i respectively in which Y_i is considered. Then if S'_i is a part of S_i , T'_i is a part of T_i ; and if T'_i is a part of T_i , S'_i is a part of S_i .

If the implications are immediate, LXIII reduces to LXI, and LXIV has no significance.

To prove Theorem XVIa we require the postulates:

LXV. Either (1) each unit A_i of the class A possesses the property or condition b_i of the scale b throughout the interval T_i , or (2) some unit A_i of the class A does not possess b_i throughout T_i .

LXVI. It is impossible that (1) and (2) of LXV should both be true. These are extensions of XLVIII and XLIX.

56. Theorem XVa. *If $X \cdot Y$ and $Y \cdot Z$, then $X \cdot Z$.*

Proof. (1) Let X_i denote any proposition of X . Then $X \cdot Y$ determines Y_i , the proposition of Y which is coupled with X_i ; and $Y \cdot Z$ determines Z_i , the proposition of Z which is coupled with Y_i . The classes X, Y and Y, Z are in one to one correspondence. Hence X, Z are in one to one correspondence, and Z_i is coupled with X_i (XVIII, XIX). Thus (I) of Def. 102 is satisfied.

(2) Let X_i be true in the interval S_{ij} . Then $X \cdot Y$ determines an interval T_{ij} , which is coupled with S_{ij} , in which Y_i is true; and $Y \cdot Z$ determines an interval U_{ij} , which is coupled with T_{ij} , in which Z_i is true. Hence U_{ij} is coupled with S_{ij} . Thus (II) is satisfied if X_i is true in S_{ij} ; and we can assert that if X_i is true in S_{ij} , then Z_i is true in U_{ij} .

(3) Let X_i be false in S_{ij} . Then $X \cdot Y$ informs us that Y_i is either true or false in T_{ij} . If Y_i is true in T_{ij} , then $Y \cdot Z$ informs us that Z_i is true in U_{ij} . But if Y_i is false in any part of T_{ij} , T_{ij} can be separated into intervals T_{ijk} such that (a) Y_i is either true or false throughout each T_{ijk} , (b) T_{ijk} is coupled with an interval S_{ijk} throughout which X_i is either true or false, (c) T_{ijk} is coupled with an interval U_{ijk} throughout which Z_i is either true or false (LXIII). Then U_{ijk} is coupled with S_{ijk} (XIX). Moreover, by LXIV, S_{ijk} is a part of S_{ij} ; and because X_i is false in S_{ij} , it is false in S_{ijk} (LXII). Thus if X_i is false in S_{ij} , S_{ij} can be separated into intervals S_{ijk} throughout which X_i is false, and to each S_{ijk} corresponds a U_{ijk} throughout which Z_i is either true or false.

Therefore if $X \cdot Y$ and $Y \cdot Z$, $X \cdot Z$ is a coupled implication, and it is true.

Cor. 1. If $X \cdot Y$ and $Y \cdot Z$, then $X \cdot Z$.

Cor. 2. Let it be asserted that $X \cdot Y$ and $Y \cdot Z$. Then the theorem can be stated "Because $X \cdot Y$ and $Y \cdot Z$, therefore $X \cdot Z$ ".

Note. If $X \cdot Y$, $Y \cdot Z$ are immediate implications, the intervals T_{ij} , U_{ij} coincide with S_{ij} , and $X \cdot Z$ is an immediate implication. This is the type of inductive syllogism which is regularly used in mathematics.

57. Theorem XVIIa. *If $X \cdot Y$, then $\text{not-}Y \cdot \text{not-}X$.*

Proof. $X \cdot Y$ informs us that all the defining postulates of $\text{not-}Y \cdot \text{not-}X$ are satisfied, except (III).

If $X \cdot Y$, then each true case of X corresponds to a true case of Y ; hence a true case of X does not correspond to a false case of Y ; hence a false case of X corresponds to each false case of Y ; hence a true case of $\text{not-}X$ corresponds to each true case of $\text{not-}Y$. Hence if $X \cdot Y$, the statement in (III) of the Def. of $\text{not-}Y \cdot \text{not-}X$ is true.

Cor. 1. If $\text{not-}Y \cdot \text{not-}X$, then $X \cdot Y$.

Cor. 2. $X \cdot Y \equiv \text{not-}Y \cdot \text{not-}X$.

Cor. 3. Any coupled implication is equivalent to its contra-positive.

Note. This theorem is very frequently employed in mathematics.

E. g. "If a point lies on a curve, its coordinates satisfy the equation of the curve"; hence "If the coordinates of a point do not satisfy the equation of a curve, the point does not lie on the curve".

58. Theorem XVIa. *A coupled implication must be true or false, and it cannot be both true and false.*

Proof. Let the implication be $X \cdot Y$. It is asserted that $X \cdot Y$ is an implication, hence there are propositions X_i of the class X which are either true or false throughout intervals S_{ij} , and propositions Y_i of the class Y , which are either true or false in intervals T_{ij} ; moreover the propositions Y_i are coupled with X_i , and the intervals T_{ij} are coupled with S_{ij} . Let S_i denote the totality of the intervals S_{ij} in which X_i is true, T_i the totality of the T_{ij} which correspond to these S_{ij} . If $X \cdot Y$ is true, every proposition Y_i of the class Y is true throughout T_i . This statement can be expressed in the form "Every unit A_i of the class A possesses the property or condition b_i of the scale b throughout the intervals T_i ".

By LXV, either every unit A_i of the class A possesses b_i throughout T_i , in which case (Def. 103) $X \cdot Y$ is true; or some unit A_i of the class A does not possess b_i throughout T_i , in which case some unit A_i possesses $\text{not-}b_i$ throughout some part T_{ij} of T_i (see LXI, LXII), whence (Def. 104) $X \cdot Y$ is false. Similarly it follows from LXVI that $X \cdot Y$ cannot be true and false.

Ex. Prove Theorem XVIIa by means of XVIa.

59. Theorem XIVA. *If $X \cdot Y$, and if X_i is a true categorical proposition, then Y_i is a true categorical proposition.*

Proof. Suppose that Y_i is false in the space time interval T_{ij} , and let S_{ij} be the interval of X_i which corresponds to T_{ij} . Since X_i is a true categorical proposition, X_i is true in S_{ij} ; therefore, since $X \cdot Y$, Y_i is true in the interval T_{ij} (Def. 102). Hence Y_i is both true and false in the interval T_{ij} , which is inconsistent with Theorem XIII. Hence there is no interval in which Y_i is false; i. e. Y_i is a true categorical proposition.

Cor. If $X \cdot Y$, and if X is a class of true categorical propositions, then Y is a class of true categorical propositions.

An example of Theorem XIVA is "If a triangle is equilateral, it is equiangular; but ABC is an equilateral triangle, therefore ABC is equiangular". The syllogisms discussed in scholastic logic are usually of this type.

60. Demonstrations. As with immediate implications, the effectiveness of coupled implications is chiefly due to the fact that there are coupled implications whose truth is asserted without demonstration: *E. g.* "If (any) two lines are straight and equal, they can be made to coincide."

Mathematical demonstrations are often presented in the informal manner described in §38. We may wish to express some part of such a demonstration in a formal manner, to make use of an inductive theorem. To do this it is sometimes necessary to separate the statement into several parts. Thus the statement "The bisector of the vertical angle of an isosceles triangle bisects the base at right angles" is equivalent to the three following propositions: "There is a straight line which bisects the vertical angle of an isosceles triangle", "If a straight line bisects the vertical angle of an isosceles triangle, it bisects the base", "If a straight line bisects the vertical angle of an isosceles triangle, it is at right angles to the base".

It is natural to enquire whether we can extend hypothetical logic by employing implications which involve implications (other than Theorems XIV, XV, XVII, which are "true in themselves"). The necessary logical processes can be developed, but there do not appear to be any implications involving implications whose truth is asserted without demonstration. Hence such a theory would not enable us to demonstrate categorical propositions or ordinary implications which could not be demonstrated without this theory; (for no additional hypotheses would be introduced).

61. Analytic Logic. The fundamental principles and processes of categorical logic are expressed by Theorems VIII to XI of Sect. V, and those of inductive logic by the theorems of Sect. VI. We can assert that these are true, and investigate whether more comprehensive definitions of class, number, implication, *etc.* are consistent with them. We can also investigate whether other logical theorems can be demonstrated from these principles and processes. These investigations, and the resulting theory, constitute one branch of Analytic Logic.

The discussion of analytic logic is beyond the scope of this monograph.

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A NEW SPECIES OF HYGROPHORUS

By L. H. PAMMEL

From the Department of Botany, Iowa State College.

Accepted for publication December 1, 1927.

Some years ago Mr. F. W. Paige of Webster County, Iowa, came to the writer to determine several species of fleshy fungi for him. Comparatively few could be recognized and identified. It was suggested to Mr. Paige that he make a thorough study of this group of plants in Webster County, Iowa, and by the means of various books which had been suggested and by exchanging specimens these species could be determined. Mr. Paige became an industrious student of this group of plants. He collected some four hundred species, and many of these were tested as to their edible qualities. The contribution immediately following gives a summary of his results. The collection of fungi made by Mr. Paige has been presented to Iowa State College, likewise his collection of flowering plants made in Alaska, and Webster County, Iowa.

One of the fungi collected has been found to be a new species of *Hygrophorus*. It has been named in honor of Mr. Paige.

Hygrophorus paigei, n. sp.

PILEUS 3-10 cm. broad, expanded-plane, and frequently repand and irregular. Egg-yellow, viscid, not at all virgate. FLESH thick, firm, thin on margin. Pallid. GILLS arcuate-decurrent, thick, subdistant, yellowish attenuate at both ends, pruinose. STEM 3-8 cm. long, subequal or tapering downward, attenuated at base, often curved, rigid, 8-16 mm. thick above, at first with an appressed, glaucous silkiness, glabrescent, innately fibrous and shining, solid refusecent within and without. SPORES narrowly ellipticlanceolate to ovate, smooth, 7-9 x 4 micr., white. ODOR none. TASTE mild.

Gregarious. On the ground among fallen leaves in frondose woods. October. After heavy frosts.

Type deposited in herbarium, Department of Botany, Iowa State College.

A LIST OF FLESHY FUNGI FROM WEBSTER COUNTY, IOWA

By F. W. PAIGE¹

From the Department of Botany, Iowa State College.

Accepted for publication December 1, 1927.

The fleshy fungi are least known of the flora of the State of Iowa. It seemed to me desirable that a study should be made of this group of a limited area in the state. I, therefore, began a study of these plants about ten years ago. I have been intensely interested in the collection and study of the same.

Webster County affords unusual opportunities for the study of saprophytic fungi of central Iowa. The county is intersected by the most important stream in Iowa, the Des Moines River, and numerous small creeks, like the North and South Lizard Creek, Prairie Creek, Deer Creek, Badger Creek, Brushy Creek, Holiday Creek, Soldier Creek, Mill Creek, Skiller Creek, and East and West Buttrick Creek.

The Des Moines has cut a deep valley, as have the other streams, in many cases with precipitous bluffs covered with trees; in some cases with small, narrow, box-like canyons that end rather abruptly on the prairie or timbered flats. The woods at the Sanitarium have been particularly valuable for the collection of these fungi. There is also prairie adjacent to Fort Dodge, but this is generally poor for the collecting of fleshy fungi.

The lowlands along the Des Moines consist of such trees as the American elm (*Ulmus americana*), cotton wood (*Populus deltoides*), soft maple (*Acer saccharinum*), boxelder (*Acer negundo*), black willow (*Salix nigra*), almond leaved willow (*Salix amygdaloides*), sandbar willow (*Salix fluviatilis*), black walnut (*Juglans nigra*), green ash (*Fraxinus lanceolata*), and other types.

The vegetation of the upland woods consists of the basswood (*Tilia americana*), white ash (*Fraxinus americana*), butternut (*Juglans cinerea*), slippery elm (*Ulmus fulva*), corky bark elm (*Ulmus racemosa*), hard maple (*Acer nigrum*), hop horn-bean (*Ostrya virginiana*), blue beech or ironwood (*Carpinus caroliniana*), moosewood (*Direa palustris*), Washington thorn or redhaw (*Crataegus punctata*), choke cherry (*Prunus virginiana*), black cherry (*Prunus serotina*), wild crab (*Pyrus ioensis*), American plum (*Prunus americana*), pin cherry (*Prunus pennsylvanica*), hazelnut (*Corylus americana*), white, red and bur oaks (*Quercus alba*, *Q. rubra*, and *Q. macrocarpa*), shellbark hickory (*Carya ovata*), and pignut hickory (*Carya cordiformis*). All of these trees have a relation to the mushrooms and toadstools found in this area.

In the identification of the species I have been in correspondence with numerous authorities on the subject, and have consulted freely the works in the appended list.

In the arrangement of the groups I have followed the best American authorities on the subject, and the general outline of arrangement of groups is that of Engler and Gilg, *Syllabus der Pflanzenfamilien*, 7th ed., pp. 37-67.

¹In the preparation of this paper I am indebted to Dr. L. H. Pammel of Iowa State College for many suggestions and for looking over the manuscript.

ASCOMYCETES

PEZIZALES

Pezizaceae

Aleuria aurantia (Pers.) Fuckel. Rare. Fort Dodge. Edible.

Peziza badia Pers. Quite common. Fort Dodge. Edible.

odorata Pk. Rare. Fort Dodge. Edible.

Helotiaceae

Sarcoscypha coccinea (Scop.) Sacc. Rare. Of reddish color. Fort Dodge. March and later.

floccosa (Schw.) Sacc. Rare. Fort Dodge. July.

HELVELLALES

Helvellaceae

Helvella crispa Fr. Rare. In woods north of Sanitarium, Fort Dodge. Edible.

elastica Bull. Olson's Park, Fort Dodge. Middle June. Edible.

lacunosa Afzel. Olson's Park, Fort Dodge. Edible.

sulcata Afzel. Rare. Olson's Park, Fort Dodge. Middle June.

Morchella conica Pers. Common. In upland woods. Gypsum Hollow. Fort Dodge. Latter part of May. Edible.

esculenta Pers. Very common. On white ash. Fort Dodge. May. Edible.

semilibera D. C. Not common. Only few were found. Fort Dodge. May. Edible.

HYPOCREALES

Hypocreaceae

Hypomyces lactifluorum Schw. Quite common on various mushrooms, especially on *Lactarius*. Woods. Fort Dodge.

SPHAERIALES

Xylariaceae

Xylaria polymorpha Grev. Common on wood, especially oak. Olson's Park, Fort Dodge, and Dolliver Memorial Park, Webster County.

var. On woods. Not common. Found especially on rotten logs. Fort Dodge.

PROTOBASIDIOMYCETES

Auriculariaceae

Hirneola auriculata Berk. Not common. Only specimen found. In woods. North of Sanitarium. Fort Dodge.

Tremellaceae

Tremella albidula. Rare. In woods. At Sanitarium, Fort Dodge.

fuciformis Berk. Rare. Found in only the one place. In woods north of Sanitarium, Fort Dodge.

frondosa Fr. More or less common. In woods.

intumescens Eng.-Bot. Not common. In woods. Fort Dodge.

lutescens Pers. Not common. In woods. Fort Dodge.

AUTOBASIDIOMYCETES

Dacryomycetaceae

Guepinia spathularia Fr. Rare. In Fort Dodge.

Thelephoraceae (None of this genus is edible.)

Stereum hirsutum Fr. In woods. Fort Dodge.

oaksii C. G. Lloyd. Rare. In woods. Fort Dodge.

rubiginosum C. G. Lloyd. Rare. Fort Dodge.

sericeum Schw. Rare. Fort Dodge.

spadiceum Fr. In woods. Fort Dodge. Common.

versicolor Sw. Fr. In woods. Fort Dodge.

Thelephora schweinitzii Fr. Very common in woods. Sanitarium and Olson's Park, Fort Dodge.

Craterellus cornucopioides (L) Pers. Rare. In woods in open places. Fort Dodge.

Clavariaceae

Clavaria cinerea Bull. Not common. In woods. North of Sanitarium, Fort Dodge. Latter part of June.

cristata Pers. Not common. In woods. North of Sanitarium, Fort Dodge. Middle July. Edible.

densa Pk. More or less common. In woods. North of Sanitarium, Fort Dodge. Latter part of June. Edible.

formosa Pers. Not common. In woods. Fort Dodge. Latter part of September. Edible.

mucida Pers. In woods. North of Sanitarium, Fort Dodge.

pistillaris L. Not common. In woods. Fort Dodge. Latter part of September. Edible.

pyxidata Pers. Not common. In woods. North of Sanitarium, Fort Dodge. Middle August. Edible.

stricta Pers. In woods. North of sanitarium, Fort Dodge. Not common. Edible.

vermicularis Scop. Not common. In woods. North of Sanitarium, Fort Dodge. Middle August. Edible.

Hydnaceae

Hydnum coralloides Sco. Very rare. In woods. Dolliver Memorial Park, Webster County; also on the bluffs west of Fort Dodge one mile.

ochraceum Fr. Rare. In woods. Fort Dodge.

pulcherrimum B & C. Rare. In woods. Fort Dodge. Very beautiful fungus. Early August.

repandum Linn. Rare. In woods. Fort Dodge.

septentrionale Fr. Rare. In woods. Fort Dodge. Middle September.

spongiosipes Pk. Rare. In woods. Fort Dodge. Early July.

Irpex lacteus Fr. Common. On small dead limbs of oak, ash, basswood,

etc. In woods. North of Sanitarium, Fort Dodge, and Dolliver Memorial Park, Webster County.

pachydon C. G. Lloyd. Not common. In woods. Fort Dodge.

tulipifera Schw. Not common. In woods. Fort Dodge.

Polyporaceae

Merulius tremellosus Schrad. Common on old wet logs. Near Big Springs west of Des Moines River and in woods Dolliver Park, Webster County, in wet places.

Fomes applanatus Pers. Common on wood such as oak, elm, Dolliver Memorial Park, Webster County, Olson's Park, Fort Dodge, and Wildcat Cave, Webster County.

everhartii E & G. Not common. Woods. Fort Dodge.

fomentarius L. Common. In woods. Fort Dodge.

fraxinophilus Pk. Not common. Found on ash. In woods. Fort Dodge.

lobatus Schw. Not common. In woods. Fort Dodge.

pomaceus C. G. Lloyd. Common. In woods. Fort Dodge.

Polyporus adustus Willd. Very common. Found on dead wood. In woods, Fort Dodge.

arcularius Batsch. Quite common. In woods. Fort Dodge.

berkeleyi Fries. Not common. In woods. Fort Dodge.

brumalis Pers. Not common. In woods. North of Fort Dodge.

cinnabarinus Jacq. Very common. On cherry and oak. In woods. North of Sanitarium, Fort Dodge.

coruscans Fries. Not common. In woods. Fort Dodge.

cristatus Pers. var *flavo-virens*. Rare. In woods. Fort Dodge.

frondosus Dickson. This species is not common. Large and more or less bushy in appearance.

fumosus Pers. Quite common. On dead wood. Fort Dodge.

gilvus Schw. Common. On rotten wood. Fort Dodge.

heteroclitus Fr. Rare. North of Fort Dodge in low woods on Des Moines River; also in Olson's Park. On ground. The fungus has rhizomes which are commonly mistaken for roots.

hispidus Bull. Rare. In wood. Fort Dodge.

perplexus Pk. Rare. In woods. North of Sanitarium, Fort Dodge.

picipes Fr. Found on old logs. Rather plentiful. In woods. North of Sanitarium, Fort Dodge.

radicatus Schw. Fairly common. Has a long stipe resembling a root. Fort Dodge.

resinosus Schrad. Found in big ravine north of Sanitarium, Fort Dodge. Common.

sessilis Mass. Not common. In woods. Fort Dodge.

spumens Sow. Found only once. In woods. Fort Dodge. (This species was sent to Dr. Murrill for identification.)

- spraguei* B & G. Not common. In woods. Fort Dodge.
- squamosus* Hudson. Not very common. In woods, Fort Dodge.
- sulphureus* Bull. Common; on oak and ash. Dolliver Memorial Park, Webster County, and Fort Dodge.
- umbellatus* Pers. Large bushy mushroom. Not common. In woods. North of Sanitarium, Fort Dodge.
- veriformis* Pk. Not common. In woods. Fort Dodge.
- vulgaris* Lloyd. Not common. In woods. Fort Dodge.
- Polystictus biformis* Fr. Rare. In woods. Fort Dodge.
- conchifer* Schw. This is a small fungus about the size of a ten cent piece in the shape of a shell. More or less common. On dead wood. In Dolliver Memorial Park, Webster County, and woods north of Sanitarium, Fort Dodge.
- hirsutus* Fr. On dead wood. Dolliver Memorial Park, Webster County.
- pergameneus* Fr. This is a small fungus found in great quantities on dead logs. Of more or less purplish color. Fort Dodge.
- versicolor* Fr. This is the most common of the group. On dead wood. Dolliver Memorial Park, Webster County.
- sanguineus* L. Not common. On dead wood. Fort Dodge.
- Trametes milicola* P & C. Not common. In woods. Fort Dodge.
- peckii* Kalchb. (synonym for *T. hispida*). More or less common. Fort Dodge.
- rubescens* Fr. Common. In woods on different species of trees. Fort Dodge.
- Daedalea confragosa* Bolton. Very plentiful. On live trees. Gypsum Hollow, Fort Dodge, and Dolliver Memorial Park, Webster County. On willows especially.
- unicolor* Bull. Common on stumps. Sanitarium north of Fort Dodge.
- Favolus canadensis* Klotzsch. Common. In woods. Fort Dodge.
- Lenzites betulina* Fr. Not common. In Gypsum Hollow, Fort Dodge.
- vialis* Pk. Quite plentiful. On rotten stumps. Gypsum Hollow and at Fort Dodge.
- Boletinus porosus* Berk. More or less common. In woods north of Sanitarium, Fort Dodge.
- Boletus affinis* Pk. Not common. Woods. Fort Dodge. August. Edible.
- badiceps* Pk. Not common. Found in Snell's woods, Fort Dodge.
- castaneus* Bull. One of the small species of Boletus. Not common. In woods. Fort Dodge. Late June. Edible.
- clintonianus* Pk. Not common. In woods. North of Fort Dodge. Edible.
- edulis* Bull. Common. In woods. North of Sanitarium, Fort Dodge, in deep ravine. One of the large species. Late August. Edible.

- edulis* Bull. var. *clavipes*. Quite common. In woods. Fort Dodge. Early July. Edible.
- glabellus* Pk. Small. In woods. Fort Dodge. Early September.
- luridus* Schaeff. Not common. Woods. Fort Dodge. Early July. Very poisonous.
- separans* Pk. Not common. Snell's woods. Fort Dodge. August. Edible.
- speciosus* Frost. In woods. North of Sanitarium, Fort Dodge.
- sphaerosporus* Pk. In woods north of Sanitarium. Nearly as large as *B. edulis*. The only *Boletus* with round spores. This species is rather plentiful. Middle June. Not very agreeable to eat.
- Strobilomyces strobilaceus* Berk. A very characteristic fungus standing out by itself. Quite common. Fort Dodge.

Agaricaceae

Cantharelleae

- Cantharellus aurantiacus* Fr. var. *pallidus*. Wildcat Cave, Webster County. Somewhat rare. Middle of June. Edible.
- cibarius* Fr. Wildcat Cave, Webster County. Very rare. Middle of July. Edible.

Marasmiaceae

- Schizophyllum commune* Fr. Common on branches of oak, maple and ash. Dolliver Memorial Park and Fort Dodge, north of Sanitarium.
- Panus conchatus* Fr. Common. In Fort Dodge.
- delabatus* Pk. Not as common as *P. conchatus*.
- rudis* Fr. Common. Fort Dodge.
- Lentinus sulcatus* Berk. In woods. Fort Dodge.
- Marasmius androsaceus* Fr. Not common. In woods. Fort Dodge. Early September.
- capillaris* Morg. Very common. In woods. Fort Dodge. Early August.
- cohaerens* Fr. Fairly common. In woods. In vicinity of Fort Dodge and in Fort Dodge. Early July.
- delectans* Morg. Rather rare. In woods. Fort Dodge. Late July.
- oreades* Fr. In Olson's Park, Fort Dodge. One of the common fairy ring mushrooms. Numerous in lawns. Middle October. Edible.
- rotula* Fr. In woods. Fort Dodge. Prettiest of our mushrooms. Pure white in color. July first.
- semihirtipes* Pk. Not common. In woods. Fort Dodge. Middle October.
- velutipes* B & C. Not common. In woods. Fort Dodge. Late August.
- wynnei* B & BR. Rare. In woods. Fort Dodge. Middle October.

Lactarieae

- Lactarius controversus* Fr. In woods. Fort Dodge. Early July.
insululus Fr. In woods. Fort Dodge. Early July.
piperatus Fr. In woods. Snell's addition to Fort Dodge. Not common. Early July. Edible.
subdulcis Fr. In woods. Fort Dodge. Early July. Edible.
torminosus Fr. In woods. Snell's addition to Fort Dodge. Late August. Poisonous.
trivialis Fr. In woods. North of Sanitarium, Fort Dodge. Late August. Suspected of being poisonous.
vietus Fr. In woods. Fort Dodge. Early August. The properties are somewhat fiery, but the pungency disappears in cooking.
volemus Fr. Not common. In woods. Snell's addition to Fort Dodge. Late June. Edible.
- Russula adusta* Fr. Very common. In woods. North of Sanitarium, also Olson's addition and Snell's addition to Fort Dodge. Early September.
- albella* Pk. In woods. Fort Dodge. Not common. Middle June.
alutacea Fr. Common in woods. North of Sanitarium, Fort Dodge, and elsewhere in Fort Dodge and Webster County. Middle June. Edible.
amygdaloides Kauff. Not common. Occurs in oak woods, clay soil, especially white oak woods. Early July. Edible.
aurantialutea Kauff. Not common. In woods. North of Sanitarium, Fort Dodge. Early September. Edible.
aurata Fr. More or less common. In woods. North of Sanitarium, Fort Dodge. Small mushroom, late August. Edible.
borealis Kauff. Not common. Found several times in woods north of Sanitarium, Fort Dodge. Middle August. Edible.
brevipes Pk. Very common. In Olson's Park, Fort Dodge. One of the short stemmed mushrooms. Early September.
chamaeleontina Fr. Abundant and in many different types. Extremely variable in color and varies in size from one to two inches in diameter. Everywhere in the woods in and near Fort Dodge. Latter part of June. Edible.
citrina Gillet. In woods. Two miles north of Sanitarium, Fort Dodge. Conspicuous because of a bright yellow color and its large size. The pileus is sometimes five inches across. Latter part of August.
crustosa Pk. Not common. In woods. Fort Dodge. Latter part of June. Edible.
decolorans Fr. In woods. North of Sanitarium, Fort Dodge. Early September. Edible.
delica Fr. In woods. North of Sanitarium, Fort Dodge. Edible.
emetica Fr. In woods. North of Sanitarium, Fort Dodge. Not uncommon. Only found in woods.

- foetens* Fr. Not common. In woods. Fort Dodge. Early July.
- fragilis* Fr. In woods. Wildecat Cave, Webster County. Not common. Early September. Edible.
- integra* Fr. Common. Fort Dodge and Olson's Park. Latter part of June. Edible.
- integra* Fr. var. *rubro-tincta*. In woods. North of Sanitarium, Fort Dodge. Usually found in clay woods and plants occur on slopes of hills. Edible.
- lepida* Fr. In woods. North of Sanitarium, Fort Dodge. Not common. Edible.
- lutea* Fr. Rare. In woods. North of Sanitarium and in Fort Dodge. Late July. Edible.
- mariae* Pk. Not uncommon. In woods. North of Sanitarium, Fort Dodge. Medium size mushroom, velvety on top.
- nigricans* Fr. In woods. Fort Dodge, and Snell's addition to Fort Dodge. Edible.
- obscura* Rom. In woods. North of Sanitarium, Fort Dodge. More common than the *lepida*. Latter part of July. Edible.
- ochracea* Fr. Not common. In Snell's woods, Fort Dodge. Edible.
- ochroleucoides* Kauff. In woods. North of Sanitarium, Fort Dodge. Edible.
- ochrophylla* Pk. var. *albipes*. Common. In woods. Fort Dodge. One of the large mushrooms of a yellowish color. September 10th. This is one of the best species of the genus to eat.
- ochrophylla* Pk. Common. In woods. North of Sanitarium, Fort Dodge. Edible.
- olivacea* Fr. In woods. Fort Dodge. Middle July. Edible.
- pectinatus* Fr. Common. In woods. Near Sanitarium, Fort Dodge, and Wildecat Cave, Webster County. Late August.
- pusilla* Pk. Not common. Found only a few times in woods in Fort Dodge. Middle August. Edible.
- roseipes* Bres. Common. In woods. North of Sanitarium, Fort Dodge, and Snell's Woods. Edible.
- sanguinea* Fr. Rare. Only one specimen found. In woods. North of Sanitarium, Fort Dodge. Red on top and more or less shiny. Late September.
- sordida* Pk. Not common. In woods, especially clay soil, where the white oak (*Quercus alba*) occurs. Latter part of June.
- squalida* Pk. Very common in spring. In Olson's Park, Fort Dodge, and Dolliver Memorial Park, Webster County. After middle of June. This is suspected of being poisonous.
- tenuiceps* Kauff. This species is not common. In woods Fort Dodge. Early September. Edible.
- uncialis* Pk. In Fort Dodge. Common, but not as common as some of the other species of *Russula*. After middle of June. Edible.

virescens Fr. In clay woods. North of Sanitarium, Fort Dodge. Edible.

vitellina Fr. In woods. North of Sanitarium, Fort Dodge. Not common. Late September.

Hygophoreae

Hygrophorus conicus Pk. North of Sanitarium, Fort Dodge. Rare. Middle of June. Suspected of being poisonous.

eberneus Fr. Found at Merrill's Ford, Webster County, and in Fort Dodge. There are two forms of this. Another small form was found north of Sanitarium in woods. This is not common. Edible.

eberneus Fr. var. *unicolor*. In woods. Fort Dodge. Not as common as *H. eberneus*. Edible.

fuscoalbus Fr. var. *occidentalis*. In woods. Fort Dodge. Late September.

paigei Pammel. In woods. Fort Dodge. Grows in groups and comes up abundantly after frost.

pratensis Fr. var. *cinereus*. In woods. North of Sanitarium, Fort Dodge. Not common. Early October.

pratensis Fr. var. *pallidus*. In woods. North of Sanitarium, Fort Dodge. Not common. Middle October.

pudorinus Fr. In woods. Fort Dodge. Late September. Edible.

russula Fr. (*Tricholoma russula*). Not common. Always found in woods. I have only found it in one place and that was north of Sanitarium, Fort Dodge, in a deep ravine. Early September. Edible.

Agariceae

Coprinus atramentarius Fr. Black mushroom. Not common. Not as large as *C. comatus* and larger than *C. micaceus*. Summer. Edible.

comatus Fr. One of the common mushrooms. Generally found in lawns and parks in cities. Fort Dodge. Spring and fall. Edible.

domesticus Fr. Said to be very common, but I have not found it so. Found in dead wood and cellars especially. Fort Dodge.

ebulbosus Pk. Good sized mushroom. Fairly common in places at base of old stumps. Fort Dodge. Summer.

fucescens Fr. Small species. Common in lawns and open spaces in woods. Fort Dodge. Summer. Edible.

micaceus Fr. A common, small, yellow mushroom, found on dead stumps of elms in cities. Spring and summer. Edible.

niveus Fr. Small white mushroom. Very abundant. Scales come off readily. Late September.

squamosus Morg. Little mushroom. Fairly common. White scales. Fort Dodge. Early June. Edible.

tomentosus Fr. Not common. In Fort Dodge.

- Paneolus campanulatus* Fr. A rather rare species. Fort Dodge.
- papilionaceus* Fr. Common. Fort Dodge. Small with long stem. Plentiful in manured soil. Summer. Suspected of being poisonous.
- retirugis* Fr. Fairly common. Fort Dodge. Found in manure piles. Summer. Suspected of being poisonous.
- solidipes* Pk. Fort Dodge. Good sized mushroom on long stem. Cap several inches high. Common on manure piles. Summer. Edible.
- Anellaria separata* Karst. Not a stable mushroom. Found near cattle. Rather rare. Found only once. Southeast of Fort Dodge.
- Stropharia semiglobata* Fr. Not common. A small mushroom. Fort Dodge.
- Hypholoma aggregatum* Pk. More or less common. In woods. Fort Dodge.
- candolleianum* Fr. Very rare. Only found in Olson's Park, Fort Dodge, in open spaces.
- capnoides* Fr. A rare fungus. In woods. Fort Dodge. Late August.
- incertum* Pk. Very common. In woods. North of Fort Dodge. Edible.
- lachrymabundum* Fr. Quite common. Abundant along the side of roads in open spaces. Fort Dodge. Middle September.
- nitidipes* Pk. In woods. Fort Dodge. Not common. Late September.
- perplexum* Pk. In woods. North of Sanitarium, Fort Dodge. Usually around base of old stumps. Edible.
- populium* Britz. Much like *H. sublateritium*. Early July.
- sublateritium* Fr. More or less common. In woods. Fort Dodge. Resembles *H. perplexum*. Early October. Edible.
- velutinum* Fr. More or less common. In woods. Fort Dodge. Along gutters. Late September. Edible.
- Psathyra umbonata* Pk. This occurs in Fort Dodge. Not common. This fungus should be cultivated.
- Psilocybe cernua* Fr. Common. More so than *P. larga*. Late October.
- foenicicii* Fr. Common. In woods. North of Sanitarium, Fort Dodge.
- larga* Kauff. In woods. Fort Dodge. Late September.
- Pholiota adiposa* Fr. Very common. In woods. North of Sanitarium, Fort Dodge.
- aggericola* Pk. *retirugis*. Found in rotten wood. North of Sanitarium. Middle October.
- albocrenulata* Pk. Only found in woods in the hollows of red oak trees. Woods at Fort Dodge. Middle August.
- cerasina* Pk. Rare. On dead logs and rotten wood. In woods north of Sanitarium, Fort Dodge. One clump fifteen to twenty specimens. Late September.

- heteroclita* Fr. In woods. Fort Dodge. Early July.
howeana Pk. Rare. In woods. Fort Dodge. Early July.
johnsoniana Pk. Found at Fort Dodge. Quite common in woods.
Late September. Edible.
lutea Pk. Not common. In woods. Fort Dodge. Early July.
ornella Pk. In woods. Fort Dodge. Not very common. Late July.
praecox Pers. Not common. Numerous only in spring. Near Wild-
cat Cave in timber. Edible, but not very good.
subsquarrosa Fr. On logs. Merrill's Ford, Fort Dodge. One of
the best of our mushrooms.
unicolor Fr. Not common. Woods. Fort Dodge. October.
Cortinarius atkinsonianus Kauff. Found in timber in soil with a good deal
of humus. Woods at Fort Dodge. Edible.
autumnalis Pk. In woods. North of Sanitarium, Fort Dodge.
Early October.
castaneus Fr. In woods. North of Sanitarium, Fort Dodge. Early
June.
elegantoides Kauff. In woods. North of Sanitarium, Fort Dodge.
Early October.
squarrosus Clem. In woods. North of Sanitarium, Fort Dodge.
Middle July.
Inocybe asterospora Quel. In woods at Fort Dodge. Early August.
fastigiata Bres. In woods. North of Sanitarium, Fort Dodge. Rare.
Late September.
fibrosa Bres. Common. In woods. North of Sanitarium, Fort
Dodge. Middle July. Poisonous.
rimosa Fr. In woods. Fort Dodge. Middle August.
Hebeloma abidulum Pk. Around Fort Dodge. Early October.
fastibile Fr. In woods. Fort Dodge. September.
hiemale Bres. In woods. Fort Dodge. Late October.
illicitum Pk. Not common. In woods. Fort Dodge. Middle Sep-
tember.
nesophaeum Fr. In woods. Fort Dodge and Webster County. Late
October.
parvifructum Pk. Not common. In woods, Fort Dodge. Middle
October.
sinapizans Fr. One of the largest of the genus. Rare. In woods.
Fort Dodge. Late June.
simile Kauff. In woods. Fort Dodge. Early October.
Flammula carbonaria Fr. Found at Merrill's Ford, Fort Dodge. Small
mushroom with slimy surface. Middle of July. Edible.
flavida Fr. Woods. North of Sanitarium, Fort Dodge. Very rare.
Edible.
lubrica Fr. This is one of the greasy mushrooms and is covered with
slime. In woods. North of Sanitarium, Fort Dodge.
Middle July. Edible.

- polychroa* Berk. In woods. North of Sanitarium and in Olson's Park, Fort Dodge. Early August.
- rigida* Pk. A very rare species. In woods. North of Sanitarium, Fort Dodge. Late September. Edible.
- spumosa* Fr. Rare. In woods. North of Sanitarium, Fort Dodge. Middle October.
- Galera tenera* Fr. A small mushroom found in woods. North of Sanitarium, Fort Dodge.
- vitaeformis* Fr. In woods. North of Sanitarium, Fort Dodge. Middle June.
- Naucoria lignicola* Pk. On dead wood. Rather rare. Fort Dodge. Late July.
- Crepidotus croceotinctus* Pk. Quite common. On wood. Not very good to eat.
- dorsalis* Pk. In woods. North of Sanitarium, Fort Dodge. Not very good to eat.
- fulvotomentosus* Pk. In woods. Fort Dodge. Early June.
- haerens* Pk. In woods. North of Sanitarium, Fort Dodge. Early August. Non-poisonous.
- herbarum* Pk. In woods. North of Fort Dodge, near Sanitarium. Early October. Not very good to eat.
- malachius* B & C. In woods. Fort Dodge. Early June.
- mollis* Fr. In woods. Fort Dodge. Early October.
- putrigens* B & C. In woods. North of Sanitarium, Fort Dodge. Late September. Not poisonous.
- sepiaris* Pk. In woods. Fort Dodge. Late June.
- Volvaria bombycina* Fr. Not common. In woods. North of Sanitarium, Fort Dodge. Beautiful large mushroom found on rotten logs. Locality has disappeared. Middle July. Edible.
- gloicephala* Fr. A few specimens on west side of Des Moines River in open woods on ground. Early July. Poisonous.
- hypopithys* Fr. In woods on rotten logs and in cattle pastures. Fort Dodge. Late August.
- speciosa* Fr. Numerous in open fields. North of Sanitarium, Fort Dodge. Late August.
- umbonata* Pk. Not common. In woods. Fort Dodge. Early September.
- Pluteus cervinus* Fr. In woods. Fort Dodge.
- cervinus* Fr. var. *albipes*. In woods. Fort Dodge. White form. Edible.
- cervinus* Fr. var. black brown form. In woods. Fort Dodge. Edible.
- cervinus* Fr. var. *petasatus*. In woods and in pastures on rotten logs. Fort Dodge. Edible.
- (All these varieties of *Pluteus cervinus* have the same habitat.)

- granularis* Pk. Very rare. In woods. Fort Dodge. Late September.
- longistriatus* Pk. Not common. In woods. Fort Dodge. Early July.
- salicinus* Fr. Rather rare. In woods. Fort Dodge. Late June.
- Entoloma clypeatum* Fr. In woods. Fort Dodge.
- grayanum* Fr. Found on golf course at edge of a grove. This species was identified by W. A. Murrill. Middle May.
- griseum* Pk. In woods. Fort Dodge near Sanitarium. Late July.
- niderosum* Fr. In woods. North of Sanitarium, Fort Dodge. Middle October.
- peckianum* Berk. In woods. Fort Dodge north of Sanitarium. Middle June.
- rhodopolium* Fr. In woods. Fort Dodge. Middle July.
- sericeum* Fr. In woods. Near Sanitarium, Fort Dodge. Middle September.
- strictius* Pk. Not common. In woods. Fort Dodge. Middle October. (There are some good mushrooms in this genus, but there are others dangerous to eat and it is best to leave them alone.)
- Clitopilus abortivus* B & C. Rare. In woods. Fort Dodge. Found only once. Edible.
- caespitosus* Pk. A rather rare fungus. Late September.
- orcella* Fr. Not numerous. In Snell's addition to Fort Dodge.
- prunulus* Fr. Not common. In woods. Fort Dodge. Late September.
- Claudopus nidulans* Fr. In woods. Fort Dodge. Rare. Middle November.
- Amanita cothurnata* Atk. In woods. North of Sanitarium, Fort Dodge. Quite plentiful. Middle July. Suspected of being poisonous.
- elongata* Pk. In woods. North of Sanitarium, Fort Dodge. Latter part of September.
- flavoconia* Atk. Rare. In woods. Near Fort Dodge. Latter part of July. Probably poisonous.
- frostiana* Pk. At Wildcat Cave and north of Sanitarium, Fort Dodge. Latter part of July. Non-edible.
- phalloides* Fr. (white form) Only found in two places. In Fort Dodge and North of Sanitarium. Deadly poisonous.
- russuloides* Pk. In woods. North of Sanitarium, Fort Dodge. Suspected of being poisonous.
- velutipes* Atk. In woods. North of Sanitarium, Fort Dodge. (Amanita contains a number of the most deadly poisonous toad stools, and it is best not to use any of them for food.)
- Amanitopsis vaginata* Fr. var. *alba*. In Olson's Park, Fort Dodge. Edible.
- vaginata* Fr. var. *fulva*. Common. In Olson's Park, Fort Dodge. Edible.

- vaginata* Fr. var. *livida*. Very common. In Olson's Park, Fort Dodge. Edible.
- volvata* Pk. Not plentiful. Frequently found growing along cement sidewalks. Fort Dodge. Poisonous.
- Lepiota acutaesquamosa* Fr. In woods. Fort Dodge. Scarcely to be distinguished from *L. friesii*.
- cepaestipes* Fr. In woods. North of Fort Dodge. Rare. Latter part of August. Edible.
- clypeolaria* Fr. More or less common. In woods. North of Fort Dodge. Latter part of September. Suspected of being poisonous.
- cristata* Fr. Not common. In woods. Fort Dodge. Middle of July.
- felina* Fr. Quite common. In woods. North of Fort Dodge. Latter part of September.
- friesii* Lasch. In woods. On outskirts of Fort Dodge. Edible, but of poor quality.
- morgani* Pk. Found in open places in lawns, fields and prairies. Fort Dodge. Poisonous. (Some people are poisoned and some are not.) Middle to latter part of September.
- naucinoides* Pk. In woods. Fort Dodge. Common, but never plentiful. More scattered. Edible.
- naucinoides* Pk. var. *squamosa*. Not common. In woods. North of Sanitarium, Fort Dodge. Does not differ much from *L. rubrotincta*. Edible.
- procera* Fr. Abundant. Fairgrounds woods in north part of Fort Dodge. Edible.
- rubrotincta* Pk. In woods. North of Sanitarium, Fort Dodge. Not common, but scattered. Also in woods in north part of city.
- Armillaria mellea* Fr. Very common. In woods. Fort Dodge; also north of Sanitarium, Dolliver Memorial Park, Webster County, and Olson's Park. Edible.
- Pleurotus corticatus* Fr. In woods. Fort Dodge. Late October.
- fimbriatus* Fr. var. *regularis*. Not common. Found only once. In woods. Fort Dodge. Middle October.
- mastrucatus* Fr. In woods. Fort Dodge. Middle August.
- ostreatus* Fr. Quite common. Everywhere on rotten wood. Fort Dodge. Summer and fall. Edible.
- petaloides* Fr. Small mushroom. Occurs on wood. Not common. In woods at Fort Dodge. Late July.
- sapidus* Kalchb. In woods. Fort Dodge. Not as abundant as *P. ostreatus* or *P. ulmarius*.
- subariolatus* Pk. In woods. Fort Dodge. Early October.
- subpalmatus* Fr. Rather rare. Dolliver Memorial Park, Webster County. Late September.
- ulmarius* Fr. Common on boxelders; sometimes also on American elms. North of Sanitarium, Fort Dodge. Fall. Edible.

- Tricholoma album* Schaeff. In woods. Fort Dodge. Common. Early October. Unpalatable.
- brevipes* Fr. In woods. Fort Dodge. Middle October.
- cinerascens* Fr. In woods. Fort Dodge. Rather common. Late October. Edible.
- grave* Pk. In woods. Fort Dodge. Early November.
- melaleucum* Fr. In woods. Fort Dodge. Early October.
- nobile* Pk. City limits of Fort Dodge. Snell Place. Early October. Suspected of being poisonous.
- personatum* Fr. Common. In woods. North of Sanitarium, Fort Dodge. Under leaves. Also found at other points in woods at Fort Dodge. Found especially in late fall. Edible.
- sejunctum* Fr. In woods. Fort Dodge. Late fall. Edible.
- terreum* Fr. In woods. Fort Dodge. Edible.
- terreum* Fr. var. *fragans*. In woods. Fort Dodge. This variety is fragrant. Middle September.
- transmutans* Pk. Common. In woods. Fort Dodge. Early September. Edible.
- Clitocybe adirondackensis* Pk. Not common. Found among dead leaves. In woods. North of Sanitarium, Fort Dodge. Edible.
- cartilaginea* Burf. In woods. Fort Dodge. Common.
- catina* Fr. In woods. North of Sanitarium, Fort Dodge. Middle August.
- compressipes* Pk. In woods. Fort Dodge. Late September.
- decastes* Fr. In Fort Dodge. Not common.
- eccentrica* Pk. In woods. Fort Dodge. Late August.
- fumosa* Pers. In woods. Fort Dodge.
- illudens* Schw. In woods. North of Fort Dodge. Found in two places. Rather common. Grows on decaying stumps and rotten logs, probably oak. This is a large mushroom. Poisonous.
- infundibuliformis* Fr. Common. In woods. North of Fort Dodge. Edible.
- laccata* Fr. In woods. Fort Dodge. Not good to eat.
- laccata* Fr. var. *striatula*. In woods. Fort Dodge. Late August. Not good to eat.
- maxima* Fr. In woods. West of Fort Dodge. Very large species. Found only once. Early July. Edible.
- media* Pk. In woods. Fort Dodge. Late September. Edible.
- multiceps* Pk. Common. Within city limits of Fort Dodge. Edible.
- nebularis* Fr. In woods on ground. Fort Dodge. Middle September. Edible.
- odora* Fr. var. *anisearia*. In woods. North of Sanitarium, Fort Dodge. Late July. Edible.

- ochropurpurea* Berk. Common everywhere in woods on high ground. Edible.
- phyllophilia* Fr. In woods. North of Sanitarium, Fort Dodge. Early September.
- pithyophila* Fr. Not common. In woods. North of Fort Dodge.
- robusta* Pk. In woods. Fort Dodge.
- subzonalis* Pk. Common. Fort Dodge.
- truncicola* Pk. Rare. Fort Dodge. Early September.
- Collybia acervata* Fr. In woods. Fort Dodge west of Des Moines River. Resembles *C. dryophila*. July. Edible.
- alcalinolens* Pk. In woods. North of Sanitarium, Fort Dodge. Early August.
- dryophila* Bull. In woods. Fort Dodge. In leaf mould especially. Does not grow thickly; more or less isolated. Late May. Edible.
- platyphylla* Fr. Not uncommon. On rotten wood. In woods. Fort Dodge. Not edible.
- radicata* Fr. Common. In woods and yards. Fort Dodge. Summer. Edible.
- velutipes* Fr. Found growing in woods in great clumps. Fort Dodge. Not edible.
- Mycena atroalba* Fr. In woods. Fort Dodge. Middle September.
- galericulata* Fr. Rather common. In woods. Fort Dodge. On rotten wood. Scarcely edible.
- haematopa* Fr. Non-poisonous, but scarcely worthwhile to collect. Middle September.
- inclinata* Fr. Non-poisonous, but scarcely worthwhile to collect. Fort Dodge. Middle June.
- leajana* Berk. Non-poisonous, but scarcely worthwhile to collect. Fort Dodge. Early September.
- parabolica* Fr. Non-poisonous, but scarcely worthwhile to collect.
- polygramma* Fr. Non-poisonous, but scarcely worthwhile to collect. Fort Dodge. Early June.
- Omphalia olivaria* Pk. In woods. Fort Dodge. Late August.
- Psalliota arvensis* Fr. Not common. In open fields. Edible.
- campestris* Fr. var. *vaporarius*. Common in greenhouses. Purely domestic fungus. Fort Dodge. Edible.
- micromengetha* Pk. Fort Dodge. Edible.
- placomycetes* Pk. Common. Fort Dodge. Edible.
- rodmanii* Pk. Along streets of cities near paving and sidewalks. Edible.
- silvicola* Vitt. Very common in pastures. Fort Dodge. Middle June.
- subrufescens* Pk. In woods. North of Sanitarium, Fort Dodge. Rather large mushrooms of reddish color. Middle July. Edible.

sylvatica Schaff. In woods. Fort Dodge. Not common. June.
Edible.

GASTEROMYCETES

Phallaceae None of this family edible.

Phallus impudicus Linn. Common. In lawns and strawberry beds. Fort Dodge.

Dictyophora ravenellii (B & C) Burt. Common in grasses and woods.
Only found on the west side of the river, Fort Dodge.

Mutinus bovinus Morgan. Rare. In woods. Fort Dodge.

Lycoperdaceae

Lycoperdon constellatum Fr. Not common. Fort Dodge. Middle September. Edible.

elongatum Berk. Common. On ground in woods. Fort Dodge.

exipuliforme Scop. Rare. Fort Dodge. Summer and fall. Edible.

gemmatum Batsch. Very common. Most common of all the puff balls. Scales fall off readily. Fort Dodge. Summer and fall. Edible.

glabellum Pk. This puff ball is usually gregarious. One or two inches in diameter. Not common. Fort Dodge. Middle September. Edible.

molle Pers. Olson's Park and woods north of Fort Dodge, and Dolliver Memorial Park, Webster County. Summer and fall. Edible.

pusillum Barsch. Woods Fort Dodge. Middle August.

pyriforme Schaff. Abundant everywhere on rotten logs. Olson's Park and woods north of Sanitarium, Fort Dodge. Also Dolliver Memorial Park, Webster County. Summer and fall.

rimulatum Pk. Not common. On rotten logs. Surface shaggy and reticulately furrowed. Fort Dodge. Middle September. Edible.

turneri E & E. Not common. On rotten logs. Fort Dodge. Edible.

wrightii var. *separans* B & C. Common. In woods and open spaces. Fort Dodge. Middle October. Edible.

wrightii var. *typicum* L. On rotten wood. Fort Dodge.

Calvatia craniformis Schw. In meadows. Not common. Along Lizzard Creek, Fort Dodge. Summer and fall. Edible.

caelata Bull. Not common. In woods. Fort Dodge. Summer and fall. Edible.

cyathiformis Bose. Quite common. In woodland pastures. Woods north of Fort Dodge. Edible.

gigantea Batsch. Quite common. In pastured woods. Fort Dodge, and Dolliver Memorial Park, Webster County. Summer and fall. Edible.

Secotium acuminatum Mont. Not common. On ground in open woods. Fort Dodge.

Bovista pila B & C. Common. In open places on lawns and in meadows. Fort Dodge, Dolliver Memorial Park, Webster County. Middle May. Edible.

plumbea Pers. Common. Woods and open places. Fort Dodge and Dolliver Memorial Park, Webster County. Middle October. Edible.

Geaster saccatus Fr. Common. In woods on ground. Fort Dodge.

triplex Jungh. Common. In woods. Fort Dodge.

Nidulariaceae

Cyathus striatus Hoff. Woods. Fort Dodge.

vernicosus (Bull.) D. C. Common in greenhouses. Fort Dodge.

Crucibulum vulgare Tul. In woods two miles north of Sanitarium, Fort Dodge.

Sclerodermaceae

Scleroderma cepa Pers. Common. In open woods. Fort Dodge.

Mycenastrum spinulosum Pk. Fort Dodge.

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THE RELATION OF OXYGEN TO THE GERMINATION OF THE CHLAMYDOSPORES OF *USTILAGO ZEA* (BECK.) UNGER.

(A Preliminary Report)

By G. A. PLATZ*

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From the Department of Botany, Iowa State College.

That oxygen plays an important role in the life processes of plant cells is well known. Its relation to the germination of the spores of certain fungi has been studied by various workers. Sanford (7) found that the spores of *Actinomyces scabies* do not germinate without oxygen. Uppal (8), in studying the relation of oxygen to spore germination in some species of the Peronosporales, concluded that indirect germination of conidia, by means of zoospores, is possible in the case of *Phytophthora colocasiae*, *P. infestans*, *P. palmivora* and *P. parasitica* without the presence of oxygen, but that direct germination, by means of a germ tube, does not take place in any of these species in the absence of oxygen. He concluded further that the conidia of *Albugo candida*, *Plasmopora viticola* and *Sclerospora graminicola*, which germinate indirectly, as well as the conidia of *Peronospora parasitica* and *P. trifoliorum*, which germinate directly, require the presence of oxygen for germination. Jones (2) found that the spores of *Ustilago avenae* do not germinate in suspensions exposed to oxygen-free atmospheres.

Some spores, such as the uredospores of the rusts, germinate readily in water apparently obtaining therefrom sufficient oxygen for germination. It is a well known fact, however, that the chlamydospores of *Ustilago zea* (Beckm.) Unger do not germinate well when placed in water. Under atmospheric pressure and at 30° C., water absorbs only 2.6 per cent of oxygen by volume, according to Olsen (5). It seemed possible that this amount might not be sufficient for the germination of these spores. A series of experiments, therefore, was planned to determine the relation of oxygen to spore germination in *Ustilago zea*.

Amount of oxygen essential to the germination of the chlamydospores of *Ustilago zea*

As a preliminary experiment, an attempt was made to germinate the spores in the absence of oxygen. In this experiment, tomato juice and gelatin were used as culture media. The tomato juice was prepared by crushing ripe tomatoes to a pulp, adding water, double-distilled from glass, in the ratio of three parts of pulp to seven parts of water and filtering the solution through absorbent cotton. The gatin-medium was prepared by acidifying a 1 per cent solution of gelatin to a hydrogen-ion concentration of pH 5.5 with benzoic acid. The pores were dusted upon the surface of

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the medium in Syracuse dishes, which were subsequently placed in desiccators from which the oxygen was removed with an alkaline solution of pyrogalllic acid.

The alkaline solution of pyrogallol was prepared by mixing a solution of one part of pyrogallol by weight in three parts of water with an equal volume of a solution of one part of potassium hydroxide by weight in three parts of water. This solution, known as Berthelot's solution of pyrogallol (9), absorbs 10 times its volume of oxygen when freshly prepared. Sufficient amounts were placed in the desiccators to absorb from one and one-half to two times as much oxygen as could possibly be present in the air of the desiccators. As the cultures were kept at 30° C., the optimum temperature for the germination of the spores according to Jones (3), the absorption of oxygen was rapid enough to prevent the formation of an appreciable amount of carbon monoxide.

Readings of the amount of germination were made microscopically after 24 hours. Three representative fields in the cultures were selected, each containing from 50 to 100 spores, and the number of germinated spores in the three fields was expressed as a percentage of the entire number of spores in the three fields. The results of five trials showed that the spores did not germinate in the absence of oxygen, whereas the checks in air produced 80 per cent germination.

As it was necessary to determine the amount of oxygen essential to the germination of the spores before attempting to correlate poor germination in water with a lack of oxygen, an experiment was planned in which the spores were subjected to atmospheres containing different amounts of oxygen. In this experiment the spores were dusted upon the surface of the media in small vials, which were subsequently placed in culture chambers containing known amounts of oxygen. The air was exhausted from the chambers by means of an air pump and the oxygen, admitted into the chambers from a gas tank, was measured with gas burrettes. The cultures were kept at 30° C. for 24 hours and readings on the germination were made as previously described. The results of five trials are shown in Table I.

TABLE I. The relation of oxygen to the germination of the chlamydospores of *Ustilago zeae*.

Medium	Atmosphere	Percent germination
10% solution of gelatin	ck. in air	86.1
	5% oxygen	83.2
	4% "	20.0
	3% "	15.0
	2% "	2.0
	1% "	Trace
	No "	0.0
30% solution of tomato juice	ck. in air	81.2
	5% oxygen	75.0
	4% "	51.8
	3% "	30.0
	2% "	15.0
	1% "	10.0
	No "	0.0

By referring to Table I, it may be noted that atmospheres containing less than three per cent oxygen were not favorable for the germination of spores. In atmospheres containing five per cent of oxygen, however, the cultures on gelatin produced 3.2 per cent germination as compared with 86.1 per cent in the checks in the open air. The cultures on tomato juice showed 75.0 per cent germination, whereas the checks in open air produced 81.2 per cent. It appears, therefore, that atmospheres containing at least five per cent of oxygen are required to support germination of the chlamydospores of *Ustilago zeae* comparable to that obtained in air.

As the media used contain a certain amount of oxygen in solution, it seemed advisable to determine whether or not the spores would germinate when immersed in these media. Suspensions of the spores were made with tomato juice and with a 10 per cent solution of gelatin acidified to a hydrogen-ion concentration of pH 5.5 with benzoic acid. Small vials were filled with these suspensions and were stoppered air-tight. In other tests the spores were fixed on microscope slides with an egg albumen fixative and were immersed by placing the slides in open vessels containing the media. In no case did the spores germinate when immersed, whereas the checks, in which the spores were dusted upon the surface of the media, produced from 80 to 85 per cent of germination. The immersed spores were not permanently injured, however, as they germinated readily if placed under favorable conditions. These results indicate that the media do not contain sufficient oxygen to support germination.

Since the spores did not germinate when immersed in the culture media it seemed possible that they might sink in water and dilute concentrations of other liquid media to such an extent as to be deprived of sufficient oxygen for germination. An experiment was planned, therefore, to determine the effect of dilution of different media. The culture media used in this experiment were tomato juice prepared as previously described, a 10 per cent solution of gelatin acidified with hydrochloric acid, a 10 per cent solution of gelatin acidified with malic acid, a 10 per cent solution acidified with salicylic acid and a 10 per cent solution of gelatin to which a definite amount of calcium benzoate (0.01g. to 10.0 c.c.) was added. These media were subsequently diluted by adding distilled water which had been twice distilled in pyrex distilling-flasks. The spores were dusted on the surface of the media and the cultures were kept at 30° C. for 24 hours. Germination counts were made as described above and Table II shows the results of 10 trials.

Reference to Table II shows that when tomato juice was used as a culture medium, the spores germinated best in solutions containing at least 30 per cent of the juice. When the juice was diluted to 15 per cent or less the germination decreased in proportion to the dilution. Likewise, when gelatin was used as a culture medium, whether acidified with hydrochloric acid, malic acid or salicylic acid, to the optimum acidity for these acids, the germination decreased with the dilution. The best germination, about 80 per cent, occurred in solutions containing at least three per cent of gelatin. In dilutions containing less gelatin, the germination gradually diminished; in a one-half per cent solution only a trace of germination occurred, irrespective of the acid used. Similar results were obtained when calcium benzoate was added to different dilutions of gelatin. Cultures in which .01 g. of calcium benzoate was added to 10 c.c. of a 10 per cent solu-

TABLE II. The effect of dilution of different media upon the germination of the chlamydospores of *Ustilago zeae*.

Medium	Concen- tration	pH	Percent germi- nation
Tomato juice	30%	4.0	80.6
" "	15%	4.0	76.8
" "	10%	4.0	49.0
" "	5%	4.0	36.2
" "	2%	4.0	21.4
" "	1%	4.0	9.2
Gelatin acidified with hydrochloric acid ¹	10%	3.0	65.0
" " " " "	5%	3.0	58.0
" " " " "	3%	3.0	42.0
" " " " "	2%	3.0	28.0
" " " " "	1%	3.0	10.0
" " " " "	½%	3.0	Trace ²
" " " " malic acid ¹	10%	4.0	72.0
" " " " "	5%	4.0	67.0
" " " " "	3½%	4.0	51.0
" " " " "	2%	4.0	38.0
" " " " "	1½%	4.0	15.0
" " " " "	½%	4.0	Trace
" " " " salicylic acid ¹	10%	5.0	88.4
" " " " "	5%	5.0	74.6
" " " " "	3%	5.0	67.6
" " " " "	2%	5.0	57.4
" " " " "	1%	5.0	9.6
" " " " "	½%	5.0	Trace
Gelatin plus calcium benzoate (0.01g. to 10 c.c.) ¹	10%	6.0	82.0
" " " " " " "	5%	6.0	76.0
" " " " " " "	3%	6.0	54.0
" " " " " " "	2%	6.0	37.0
" " " " " " "	1%	6.0	12.0
" " " " " " "	½%	6.0	Trace

¹Checks of gelatin alone produced less than one per cent germination.²Trace signifies less than one per cent.

tion of gelatin produced 82 per cent of germination. When the same amount (0.01 g.) of calcium benzoate was added to 10 c.c. of more dilute solutions of gelatin, the germination decreased in proportion to the dilution, only a trace of germination showing in one-half per cent solutions.

At first thought, it may be supposed that the effect of the dilution of the culture media is due to a diminution of some nutrient substance that is necessary for the germination of the spores. The data of Table II do not confirm this supposition. Since the spores in the check cultures of gelatin alone did not germinate, there appears to be nothing in the gelatin itself favorable to their germination. Moreover, the dilution of the acid media did not produce an appreciable change in the hydrogen-ion concentration of the cultures. Thus the effect of dilution cannot be due to a change in acidity. In the cultures in which calcium benzoate was used, the same amount was added to each dilution of the gelatin. In was, therefore, not a difference in the amount of this substance added to the culture medium that produced the difference in germination. Furthermore, it has been found by Platz, Durrell and Howe (6) that good germination of these spores may be obtained on such non-nutrient media as silica jelly and

collodion. Nutrients are evidently non-essential to the germination of the chlamydospores of *Ustilago zeae* and the dilution effect must be attributed to some factor other than the diminution of nutrient substances.

It may be thought that a difference in the osmotic pressure of the various dilutions of the media used accounts for the difference in germination. By the freezingpoint method the osmotic pressure of tomato juice diluted with 70 parts of water, a dilution on which 80 per cent of the spores germinated, was found to be only three atmospheres greater than that of tomato juice diluted with 99 parts of water on which only nine per cent of the spores germinated. When one considers the magnitude of the osmotic pressure of such cells as the chlamydospores of *Ustilago zeae*, it is not probable that a difference of three atmospheres would have much influence upon their germination.

That a difference in the surface tension of the dilutions might be responsible for the difference in germination was considered. The surface tension of distilled water and of various dilutions of tomato juice was determined by the modified ring method described by Durrell, Person and Rogers (1). An average of 20 trials with each liquid is given in Table III.

TABLE III. The surface tension of various dilutions of tomato juice and of distilled water.

Medium	Dilution	Surface Tension
Tomato juice	30%	54.4 dynes
" "	15%	56.6 "
" "	10%	57.6 "
" "	5%	60.0 "
" "	2%	61.2 "
" "	1%	62.8 "
Water (twice glass-distilled)		69.2 "

As shown in Table III, the surface tension of a 30 per cent solution of tomato juice is only 8.4 dynes less than that of a one per cent solution and only 14. dynes less than that of distilled water. Noble (4) working with the spores of *Urocystis tritici* found no correlation between germination and the surface tension of the media used, though he used differences of surface tension as high as 34 dynes. It appears, therefore, that differences in surface tension ranging from 2.2 dynes to 14.8 dynes are too small to affect the germination of the spores of *Ustilago zeae* to any great degree.

Whether the spores are injured by excessive imbibition when placed in water or dilute solutions was also given consideration. The spores were fixed on microscopic slides with an egg albumen fixative. These slides were placed under the microscope and the spores were flooded with water, tomato juice solutions ranging from 30 per cent to one per cent, and gelatin solutions ranging from 10 per cent to one-half per cent. The diameter of the spores was measured before and after flooding with the aid of an eyepiece micrometer. By using a 1.8 mm. oil-immersion objective and a 25x eyepiece, the protoplasmic contents of the spores could be easily distinguished from the cell wall. No plasmolysis or excessive swelling of the spores was noted. In fact, no visible change occurred with the use of any of the liquids.

As evidence that the lowered percentage of germination in dilute solutions was the effect of a lack of oxygen, it seemed advisable to determine to

what extent the spores sink in the different media, for the greater the extent of surface exposed to the oxygen, the greater the percentage of germination should be if the oxygen is the controlling factor. Since the sinking of an object, when placed in a liquid, is proportional to the difference between its specific gravity and that of the liquid, it was necessary to find the specific gravity of the spores and of the media. The approximate specific gravity of the spores was found by making suspensions of them in liquids of known specific gravity and noting to what extent they sank in these liquids. The liquids used for these suspensions were solutions of alcohol in water with specific gravities ranging from 0.85 to 0.99, distilled water with a specific gravity of 1.00, and solutions of sodium carbonate in water with specific gravities ranging from 1.01 to 1.10. As seen by Table IV, the specific gravity of the spores ranges from 0.95 to 1.05.

In determining the specific gravity of the media, weighings with a specific gravity flask were made with water and the tomato juice solutions. The specific gravity of the gelatin solution was found with a hydrometer. The findings of these tests are shown in Table V.

TABLE IV. The specific gravity of the chlamydospores of *Ustilago zeae*.

	Specific Gravity	Status of spores
Water plus ethyl alcohol	0.95	all sank rapidly
" " " "	0.90	all sank rapidly
" " " "	0.95	almost all sank rapidly
" " " "	0.96	4/5 sank
" " " "	0.97	3/4 sank
" " " "	0.98	2/3 sank
" " " "	0.99	1/2 sank
" distilled	1.00	1/2 sank
" plus sodium carbonate	1.01	1/3 sank
" " " "	1.02	1/4 sank
" " " "	1.03	1/5 sank
" " " "	1.04	1/10 sank
" " " "	1.05	a few sank
" " " "	1.10	none sank

TABLE V. The specific gravity of various dilutions of tomato juice and gelatin as contrasted with that of distilled water.

Liquid	Specific Gravity
30% solution of tomato juice	1.0078
15% " " " "	1.0035
10% " " " "	1.0018
5% " " " "	1.0006
2% " " " "	1.0002
1% " " " "	1.0000
Check (distilled water)	0.9972
10% solution of gelatin	1.0197
5% " " " "	1.0083
3% " " " "	1.0021
2% " " " "	1.0007
1% " " " "	1.0000
1/2% " " " "	1.0000
Check (distilled water)	0.9972

By comparing the data of Tables IV and V, it may be noted that the specific gravity of a 30 per cent solution of tomato juice is 1.0078 and that of a 10 per cent solution of gelatin is 1.0197, whereas the specific gravity of water is 0.9972. It also may be noted that the specific gravity of the solution decreases in proportion to the dilution. Since most of the spores are lighter than the more concentrated solutions of the culture media, it is evident that they will not sink as deep in these as they do in water. This was confirmed by actual observation. The spores were dusted upon the surface of drops of the various solutions placed on the edge of a microscope slide. This slide was fixed in a Barber pipette-clamp so as to be readily adjusted to such a position as to obtain a focus on the spores floating on the surface of the dilution drops. By means of a Bausch and Lomb 8.5 mm. objective and a 25x eyepiece, the depth to which the spores sank in the various dilutions could be seen. In water and in the more dilute solutions of tomato juice and gelatin, the spores sink to 0.8 of their diameter. When dusted upon the surface of the more concentrated solutions, they sink to only 0.4 or less of their diameter. The difference of the exposure of the surface of

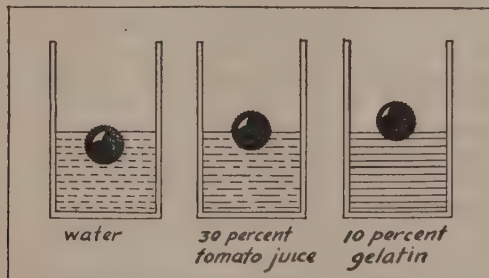


Fig. 1.

the spores is graphically shown in Fig. 1. It is possible that this difference of exposure to the normal amount of oxygen in the atmosphere is sufficient to account for the poor germination obtained when the spores are placed in water or in dilute solutions of culture media. There is not enough oxygen in the liquid to support their germination and they apparently sink to such a degree as to be deprived of sufficient oxygen from the air.

SUMMARY

The chlamydospores of *Ustilago zeae* (Beckm.) Unger do not germinate in the absence of oxygen.

The spores dusted on the surface of culture media did not germinate when placed in chambers from which the air was exhausted by means of an air pump nor when placed in atmospheres from which the oxygen was removed by the use of an alkaline solution of pyrogallie acid.

Atmospheres containing at least five per cent of oxygen are required to produce germination comparable to that obtained in the open.

When suspended in water, tomato juice or gelatin and kept in air-tight vials, the spores failed to germinate, apparently due to a lack of oxygen.

When dusted upon the surface of water and dilute solutions of liquid media, the spores do not germinate as well as when dusted upon more concentrated solutions of the media. They sink deeper in the dilute solutions than in the more concentrated and apparently are deprived of sufficient oxygen to support germination.

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ACCELERATION OF LIPASE ACTIVITY BY SUBSTANCES CONTAINING VITAMIN A

By BIRGER L. JOHNSON

(From the Laboratory of Biophysical and Physiological Chemistry, Iowa State College, Ames.)

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There has been much speculation as to the chemical function of vitamins, but little definite information is to be found in the literature. The work on record appears to be confined to the action of vitamin extracts upon enzyme activity. Thus, M. J. Villaroel (1) observed that extracts of vitamin B obtained from yeast, pancreas and testes augment catalase action of extracts of liver. These extracts were also found to accelerate castor-bean lipase and the amylolytic action of taka-diastrase and of amylase. His suggestion that the vitamins function as accelerators of enzymes is not without criticism in the literature. U. Summartino (2) found that zymase activity was accelerated significantly by vitamin extracts. With identical extracts he observed slight positive acceleration of tryptic and diastatic action but no acceleration of peptic action. Summartino, unfortunately, does not state the kind of vitamin extract with which he worked. Tanaka Yoshio (3) made the observation that the action of castor-bean lipase was accelerated by additions of water extracts of the castor-bean seed. He ascribed the acceleration to the inorganic compounds and proteose present. None of these investigators carried their work to the stage that all factors other than vitamins were eliminated.

The purpose of the work to be described is to determine whether vitamin A is a factor in the acceleration of lipase action.

THE METHOD

Preliminary experiments showed that ether-extracted commercial pancreatin powder increases the rate of hydrolysis of ethyl butyrate sufficiently so that distillable amounts of butyric acid can be obtained in the course of a few hours. Advantage of this has been taken to note the effect of increasing quantities of a vitamin A concentrate upon a medium containing pancreatic lipase.

Early in this work it was recognized that the determination of the influence of vitamin A concentrates upon lipase activity hinges upon the completeness with which the butyric acid is distilled. This factor was tested as follows: Into each of four 50 cc. balloon flasks was introduced 11 cc. of a dilute butyric acid solution and then 150 cc. of CO₂-free distilled water. The butyric acid was then distilled off as described later, a few pieces of pumice being used to prevent "bumping". When only a few cubic centimeters remained in the balloon flasks the distillation was stopped and the butyric acid titrated with 0.2117N sodium hydroxide. Phenolphthalein was used as indicator.

*This forms part of a thesis submitted to the Graduate Faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

In Table I are given data showing that with the technique used the distillation of the butyric acid is quantitative.

TABLE I. Alkali (cc. of 0.2117N NaOH) required to neutralize 1 cc. of butyric acid in distillate and in the solution before distillation.

Before distillation	Distillate
2.95	3.04
2.80	2.99
2.95	2.95
3.13	-----

To enhance the accuracy of the distillation the directions which immediately follow indicate the use of 200 cc. of distilled water for dilution in addition to the wash water that is introduced during the transfer from the digestion flask to the distillation flask. Although the distillation of butyric acid is quantitative it does not follow that the titration values of control tests should agree as closely as may be inferred from the above data. In fact such agreement would be very remarkable indeed considering the more or less heterogeneous condition of the reaction mixture which has been used in the test to be described. The general tendency of the results is deemed of most importance. This heterogeneity was overcome as much as possible in all cases by shaking the contents of each flask by hand as often as was convenient.

Preliminary work showed that a medium composed of 0.5 gms. of pancreatin taken up in 5 cc. of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ solution (1 cc. = 0.15 gms. $\text{Na}_2\text{HP}_4 \cdot 12\text{H}_2\text{O}$) readily hydrolyses ethyl butyrate. To this medium were added 0.5 cc. of ethyl butyrate and four drops of toluol as a preservative. In order to present as much surface of vitamin concentrate as possible, it was deposited from an ether solution on to a specially acid treated sea sand. Thus the variant of an experimental series was the vitamin concentrate sand preparation. After a four to eight hour digestion period, the contents of each flask was made acid with 5 cc. of sulphuric acid (10 cc. conc. H_2SO_4 and 88 cc. of water). The contents of each flask were then transferred to 50 cc. balloon flasks and diluted with 200 cc. of CO_2 -free distilled water. The balloon flasks were then connected to a Reichert-Meissl distillation apparatus. The collection of 200 cc. of distillate removed quantitatively the butyric acid from the digestion mixture. The butyric acid in each flask was then titrated with standard sodium hydroxide. The difference noted between the titer of the control and the other flasks of the series was taken as an indication of the effect of addition of vitamin concentrate upon lipase action.

EXPERIMENTAL RESULTS AND DISCUSSION

A. The effect of vitamin A concentrate from butter fat upon lipase activity.

The Sand Preparation. The method for making vitamin A concentrate the sand, according to Steenbock, Sill and Buell (4), is as follows. Special sea sand was allowed to stand for several hours in aqua regia. The mixture was filtered and washed with distilled water until free from chlorides. The sand was then dried and kept in stoppered bottles. Three hundred grams of the fat were treated with 600 cc. of a 20 per cent alcoholic potash. After four hours 2400 cc. of water were added and the solution

extracted three times with ether. The ether extracts were washed with a small volume of water and the extract evaporated on such an amount of the sand that the preparation contained 2.5 per cent of the vitamin A concentrate.

In Table II are given data showing that the above preparation does stimulate the lipase activity. The digestions were carried out for 4.5 hrs. at 30° C. About nine drops of toluol were added as a preservative.

TABLE II. Effect of vitamin A sand preparation on lipase activity.

Grams of sand preparation	No. cc. of 0.1N NaOH required to neutralize the butyric acid formed		
	I (1)	II (2)	III (3)
0	6.29	5.67	5.10
0.500	8.44	6.12	7.69
1.000	9.03	6.51	8.22
1.500	10.39	6.79	8.93

(1) 0.5 gms. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 5 cc. of water, 1 cc. of ethyl butyrate

(2) Same as above except 0.65 gms. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$

(3) Same as (2) except 0.4 cc. of ethyl butyrate used

B. The effect of factors other than vitamin A in the sand concentrates upon the lipase activity.

In order to determine whether the vitamin A is the sole stimulating factor for lipase action in the sand preparation it is necessary to study separately other constituents of the concentrate. There are five possibilities to be considered in the following order.

1. *The effect of an oil film upon lipase activity.* For this purpose a film of cottonseed oil was deposited on the sand. The preparation contained 2.5 per cent of the oil. The effect of this preparation is shown by data given in Table III. The data show that the oil film did not stimulate the hydrolysis.

TABLE III. The effect of a film of cottonseed oil on sand upon lipase activity.

Grams of activated sand	No. cc. of 0.1N NaOH required for titration	
	I (1)	II (2)
0.00	2.72	8.48
0.15	-----	8.10
0.30	2.60	7.77
0.45	-----	7.45
0.60	2.20	8.17
0.75	-----	7.88
0.90	2.15	8.40
1.05	-----	7.70
1.20	1.58	7.82

(1) Pancreatin and Na_2HPO_4 absent. Digestion 4¼ hours at 20° C.

(2) Standard method used. Digestion 5¾ hours at 30° C.

2. *The effect of sea sand alone on the lipase activity.* Data in Table IV show that the sand alone did not stimulate the hydrolysis, in fact there is a tendency toward depression.

TABLE IV. The effect of sea sand on the lipase activity.

Grams of sand	No. cc. of 0.10N Naoh required for titration
0.00	6.22
0.50	6.18
1.00	4.80
1.50	5.04

3. *The effect of toluol upon the lipase activity.* In Table V are given data showing that the addition of toluol does not increase the hydrolytic activity of the sand butter fat preparation but decreases the stimulation. The digestion period was for nine hours.

TABLE V. The effect of toluol upon the stimulating effect of butter fat preparation for lipase activity.

Flask	Sand preparation	Toluol	No. cc. of NaOH N/10 required for titration
0	0.00 gms.	2 drops	13.22
1	0.30 "	4 "	15.28
2	0.30 "	6 "	14.14
3	0.30 "	8 "	14.68
4	0.30 "	10 "	13.52

4. *The effect of cholesterol upon lipase action.* Cholesterol and lecithin are common constituents of animal fats. E. F. Terraine (5) has shown that lecithin increases the activity of pancreatic lipase only slightly. He used monobutyryn as the substrate. It would be unusual, therefore, to expect lecithin to have great stimulating power upon lipase when acting upon ethyl butyrate as substrate. Cholestrol was found to have no appreciable stimulation.

5. *The effect of sodium oleate upon lipase activity.* Commercial oleic acid was treated with sodium hydroxide in such a manner that not all the oleic acid was neutralized. The resulting soap was then allowed to dry after which the excess oleic acid was removed by several extractions with ether. A small shaving of sodium oleate and sodium palmitate were ground up in a mortar with specially treated sea sand. Tables VI & VII give the

TABLE VI. The effect of sodium oleate upon lipase activity.

Grams of vitamin A concentrate sand	No. of cc. of N/10 NaOH required to neutralize the butyric acid
0.00	13.51
0.10	12.77
0.20	13.57
0.30	16.55
0.40	15.10
0.50	16.90
0.60	16.89
0.70	16.61
0.80	18.41
0.90	17.90

data obtained. It is very apparent that the soaps increase the activity of the lipase. It may be that all soaps show this property. The pancreatin preparations used in the two experiments were not identical and evidently not of the same strengths.

TABLE VII. The effect of sodium palmitate upon lipase activity.

No. cc. Na Palmitate Soap (1)	No. of cc. of N/10 NaOH required to neutralize butyric acid
0.00	11.55
0.10	1.80
0.30	2.29
0.50	2.31
0.70	2.65
0.90	2.95
1.30	3.50
1.60	4.09
1.90	4.10

(1) Sodium palmitate app. 1 Normal. No sand.

C. The effect of vitamin A concentrates made from sources other than butter fat.

The acceleration of lipolysis noted in the previous experiments was caused by the addition of vitamin A concentrate made from butter fat. Similar acceleration should also be noted in the case of a concentrate made from cod liver oil as this substance has been shown by animal experiments to be rich in vitamin A. Similar concentrates made from cottonseed oil, lard and inactivated butter fat should prove not to stimulate lipase activ-

TABLE VIII.

Type of Vitamin A Prep.	No. cc. of N/10 NaOH required to neutralize Butyric Acid			
	Cod liver oil Vitamin A Preparation	Cottonseed Oil Vitamin A Preparation	Lard Vitamin A Preparation	Inactivated Butterfat
Incubation hrs.	4	4	4	4
Gms. sand Prep.				
0.000	3.38	3.92	3.03	3.41
0.500	4.06	3.90	3.80	4.90
1.000	5.00	5.31	5.09	4.80
1.500	5.15	4.81	4.20	4.20
2.000	5.44	5.95	3.98	4.18
Incubation hrs.	9½	10½	10	5½
Gms. sand Prep.				
0.00	4.80	4.87	5.20	4.52
0.50	7.00	7.08	7.95	6.98
1.00	6.06	6.06	7.05	4.57
1.50	6.50	-----	7.90	5.90
2.00	6.50	6.08	8.25	6.10
0.00	5.42	4.45	5.40	4.02
2.50	6.20	6.85	7.40	4.79
3.00	6.10	6.48	7.45	5.10
3.50	5.80	5.73	7.75	6.33

ity if vitamin A is the stimulant. This reasoning has been tested out. The experimental data are shown in Table VIII.

The concentrates were prepared according to the method previously outlined. The butter fat was inactivated, *i. e.*, the vitamin A was destroyed, by passing air through the butter fat at 120° C. for four hours.

It is apparent that all the concentrates stimulated the lipase activity. This is not in harmony with the vitamin A content as determined by feeding animals the fats used in the above experiments. It seemed advisable then to test the vitamin A potency toward rats of the concentrates prepared by the outlined ether extraction.

D. Rat growth studies on vitamin A potency of concentrates from cod liver oil, cottonseed oil, lard, and inactive butter fat.

The acceleration of lipase activity caused by concentrates from cod liver oil, cottonseed oil, and inactivated butter fat as shown previously in Table VIII may be assumed to be due to vitamin A if these concentrates permitted animal growth at a parallel rate.

The only condition under which the rate of animal growth would be parallel for concentrates made from cod liver oil, cottonseed oil and lard would be that the same amount of vitamin A was extracted in each case: This would imply that very nearly the same volumes of ether must be used in the extraction and that vitamin A be a substance rather insoluble in ether. This supposition has support in the fact that vitamin A cannot be readily extracted with ether from plant sources.

Rat experiments were therefore devised to determine the accuracy of this reasoning. Rats were fed a standard deficient diet until their growth was stationary, when concentrates from the various sources were added to the ration. The amount of concentrates was based upon a 5 per cent level of the original source. Control rats were fed the deficient ration plus 5 per cent of the original source.

The data obtained showed that only the concentrate from cod liver oil permitted animal growth. Steenbock, Sell and Buell (4) reported that a similar concentrate from butter fat gave growth. Addition of the concentrates made from inactivated butter fat, lard and cottonseed oil did not seem to improve the deficient ration. The fact that the animals lost weight, manifested soreness of the eyes, and eventually died is sufficient proof of this. The rats on the ration supplemented by concentrates made from cod liver oil fared somewhat better; three of the five increased their body weight, especially so after the concentrate had been added to the otherwise deficient ration more liberally, approximately to a 30 per cent level.

The rat growth experiments then indicate the following facts: Vitamin A in cod liver oil can be concentrated by the method used. Concentrates made from cottonseed oil, lard and inactivated butter fat are, as far as can be ascertained from the data, lacking in vitamin A. The increase in lipase activity by these same concentrates may be due in part to vitamin A, especially when this vitamin is present and also may be due to some other factor as yet undetermined. It may be pointed out that fatty acids are present in the vitamin concentrates as they have been prepared and that these undoubtedly constitute a source of acceleration.

E. The effect of the removal of fatty acids from vitamin A concentrates upon lipase activity.

It has previously been noted that soaps cause an increase in lipase activity. This suggests that there may have been present during the digestion period soaps or substances which might have given rise to soaps and hence caused the stimulation of the lipase noted above. Since the vitamin A concentrate was obtained by extracting with ether, a water, alcohol, potassium soap, solution there was, no doubt, ample opportunity for soaps being present in the ether extract before this was washed with water. The ether extract was, however, washed six or seven times with distilled water. The last water gave no test with AgNO_3 , showing the absence of the fatty acids. The final product was also tested for the presence of ash by igniting a small portion. No ash could be observed. (A very delicate test for the presence of alkali in the ash under these conditions is the application of phenolphthalein and a few drops of water.) It is safe, therefore, to assume that soaps were absent from the vitamin concentrate. The presence of fatty acids in the vitamin concentrate cannot, however, be precluded on the basis of these simple tests. As a matter of fact, soaps hydrolyse in water to give alkali hydroxide and fatty acids. A simple test using sodium palmitate showed that the fatty acid was readily extracted by ether from water solution.

The removal of fatty acids from vitamin concentrates has been accomplished, but with some difficulty and probably with some loss of potency. A weighed portion of the concentrate as obtained according to Steenbock, Sell and Buell (4), and outlined above, was dissolved in absolute alcohol. A drop or two of phenolphthalein was added and the fatty acids titrated with anhydrous alcoholic sodium hydroxide. From these data was calculated the required amount of alcoholic sodium hydroxide necessary to neutralize the fatty acids in a larger portion of the vitamin concentrate.

TABLE IX. Effect of fatty acid free concentrates on lipase activity.

Type of Vitamin A sand preparation	Cod Liver Oil	Cottonseed Oil	Lard	Inactivated Butter Fat	Butter Fat
Exp. No.	I	IV	V	VIII	
Gms.*					
Sand Prep.	cc. of N/10 NaOH				
0.00	2.86	2.55	3.50	2.81	
0.50	3.25		3.50	3.93	
1.00	3.40	3.60	4.12	3.65	
1.50	3.30	3.60		4.40	
Exp. No.	II	III	VI	VII	IX
0.00	3.08	2.13	2.04	1.92	1.80
0.50	3.30	2.69	2.29	2.71	1.86
1.00	3.59	2.88	2.46	2.35	2.49
1.50	3.81	2.96	2.22	2.43	2.59
2.00	3.79	4.87	2.20	2.81	2.12
0.00		1.95	2.61	2.25	2.19
2.50	3.40	3.65	2.75	2.55	2.49
3.00	3.47	2.59	2.52	2.53	2.41
3.50		3.31	2.33	2.92	2.55
0.00	3.33	1.87	2.27	2.23	1.65

*2.5% fatty acid free concentrate.

The neutralized concentrate was then evaporated by vacuum until all the alcohol and water were removed. The concentrate plus the soaps was then extracted with anhydrous ether.

Vitamin concentrates made from butter fat, cottonseed oil, lard and cod liver oil were so treated and afterward tested for stimulatory power. The results of this work are embodied in Table IX.

The concentrate used in Exp. III was shown to contain traces of fatty acids and this may account for the slightly higher results than is to be noted in Exp. IX in which a similar concentrate from butter fat was used, or the cod liver oil concentrate is richer in vitamin A. In Exp. II the concentrate was treated for the removal of fatty acids as already described and subsequently found to be free of these substances. It is to be noted in Exp. II that there was a gradual acceleration followed by a downward tendency. This is suggestive, especially in consideration of the irregular results obtained with concentrates from cottonseed oil and lard. In Table VII, it is shown that 1.90 cc. of approximately N-1 sodium palmitate gave a stimulation of 2.55 N/10 NaOH over the control. The maximum stimulation in Exp. III is 2.74 cc. It is unlikely that there were present enough fatty acids in the amount of concentrate used in Exp. III to give soaps of the same concentration as existed in the experiments with sodium palmitate. It would seem, therefore, that not all of the acceleration obtained in Exp. III was due to soaps. Exp. II bears this out.

It is interesting to note that those concentrates giving most regular acceleration of lipase activity also support animal growth.

SUMMARY

1. A method for studying the effect of vitamin A concentrates upon the enzyme lipase has been devised.
2. Several phases of this method have been studied to eliminate possible sources of acceleration other than that caused by vitamin A or similar concentrates.
3. An acceleration of lipase activity by vitamin A concentrates has been noted and studied with a view to correlating this acceleration with the vitamin A content of various fats and oils.
4. Concentrates made from butter fat and cod liver oil have been found to accelerate lipase activity. A concentrate from inactivated butter fat was found to give very little acceleration.
5. Concentrates made from cottonseed oil and lard were found to accelerate lipase activity. These concentrates, however, have been shown to contain fatty acids which react with the medium to give soaps.
6. Sodium oleate and sodium palmitate have been found to accelerate lipase activity.
7. Vitamin A concentrates from butter fat and cod liver oil have been separated from their free fatty acid content and found to give measurable acceleration of lipase.
8. Vitamin A concentrates made from lard and cottonseed oil were similarly freed of their fatty acid content and found to increase lipase activity irregularly.

9. While there is no accurate parallism between the lipase stimulating activity of the vitamin A concentrates and their anti-xerophthalmic effect upon rats, there is indication that vitamin A timulates lipase activity. Further work is required to resolve this inconsistency of results.

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NOTE ON THE DISTRIBUTION OF THERMOPHILIC SPOILAGE BACTERIA

By LAWRENCE H. JAMES

From the Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

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Spoilage of canned foods by thermophilic sporulating bacteria has been extensively studied by the research staff of the National Canners' Association. In 1920, Donk⁷ isolated and described a highly resistant thermophile which withstood a temperature of 100° C. for 17 hours or 120° C. for 11 minutes. In the same year, Bigelow and Esty² reported studies in which they showed that the spores of "typical thermophilic organisms" survived an exposure at 120° C. for as long as 22 minutes.

Studies of anaerobic thermophilic bacteria have been reported by Barlow¹ and by Damon and Feirer⁶. Their results are significant to the problem of thermophilic spoilage of foods, but limited space does not permit detailed reference to them here.

After studying 214 cultures of non-gas-forming thermophilic bacteria, Cameron and Esty⁴ divided them into two groups, one of facultative and the other of obligate thermophiles. Both types were said to produce the typical "flat sour" condition in canned foods. Extensive studies of the types of thermophilic bacteria which are significant in the high temperature spoilage of canned foods have led to the recognition of three types; namely, the "flat sour," either facultative or obligate thermophilic, and the "hard swell," or those sporulating anaerobic thermophiles which when grown in canned foods liberate sufficient gas to cause the swelling of the container to the familiar "hard swell" deformity. Since the identification of these types, Werkman and Weaver⁵ have added a fourth, which is characterized by its ability to liberate large quantities of hydrogen sulfide and for which the name *Cl. nigrificans* is proposed.

The identification of these types of thermophilic bacteria was a long step toward the elimination of the dangers of wholesale spoilage from the category of the difficulties confronting the commercial canner. Cannery surveys by Cameron, Williams and Thompson⁵ and by Cameron³ have demonstrated the effective removal of the seat of infection from cannery systems.

The elimination of these organisms from canneries where spoilage has been encountered is extremely important, but it is equally as significant to determine their natural habitat and, if possible, the means by which they may gain entrance to canning establishments. Such studies must be made in the cannery itself, where the diverse procedures under the various environmental conditions can be observed at first hand. Cameron, Williams and Thompson⁵ reported that "Tests for spoilage thermophiles on peas entering the canning plant were uniformly negative. Their presence on raw cut corn was suspected on rare occasions. Tests made upon cane sugar

showed that this substance contained definite numbers of thermophilic spores.”

In the preceding references no figures are given to show the extent of the infections. The same workers have since shown the presence of these thermophiles in stable fertilizer and on fresh peas*. With a desire to confirm some of these findings, brief investigations were made at a large corn cannery which offered opportunities for study of the various phases of the problem.

Acknowledgment is due the research staff of the National Canners' Association for cooperation by which the author was enabled to become thoroughly familiar with their methods and with media for cultivation of these spoilage thermophiles. In the present study, two modifications in technique were introduced, the use of beef infusion instead of liver infusion for the cultivation of the “hard swell” thermophiles and the removal of vegetative forms by heating at 80° C. for 20 minutes instead of boiling for 8 minutes.

As practically no spoilage had been encountered in this plant for 10 years, large numbers of the spoilage organisms were not expected. Two series of samples collected in the factory on different days showed few spoilage organisms. Each series included samples obtained from eight different sources in the cannery, beginning with the fresh corn and ending with the canned product. The results showed a total absence of “flat sour” types, the presence of a very few organisms of the *Cl. nigrificans* type, and a predominance of the thermophiles producing hard swells. Two significant points were brought to attention: first, that *Cl. nigrificans* was found only in the preheated, unprocessed corn to which sirup had been added; second, that the time and temperature used to cook the corn were sufficient to kill all the organisms capable of producing hard swells.

The different raw materials entering the plant were comparatively few in number; namely, fresh corn with possibly some soil contamination, sugar, salt, and water. Accordingly, a survey of the possible sources of the two types of spoilage organisms found was made. Samples of four different batches of sugar and salt were examined for spoilage thermophiles, with the following results:

Sugar (20 samples) 20 contained “flat sour” type
10 contained *Cl. nigrificans*
5 contained “hard swell” type

Salt contained no spoilage organisms.

The results obtained from salt are not conclusive, since these organisms are not known to be capable of growth in a salt concentration of from 0.5 to 2.5 percent, which was used in our tests.

Examination of the water used in the cannery revealed no thermophiles of spoilage types.

The results of the examinations of the sugar and salt samples did not indicate a possible source of the large numbers of the “hard swell” type found throughout the factory. Of the materials entering the cannery, the husks removed from fresh corn showed the greatest number of these organisms per gram. Our attention, therefore, was directed to the field soil and its adjuncts. Nine samples of soil and three of manure were obtained from neighboring fields, which were then furnishing the corn to the can-

*Personal communication.

nery. All the fields had been tilled for several years, and most of them had been fertilized more or less continuously.

Seven of the soil samples were from fields previously fertilized with manure and two were from fields fertilized with pea vines. Two of the manure samples were removed from piles which had cured for some months and one was of fresh material. Fifty gram subsamples were thoroughly washed in 100 c.c. of sterile water, and this solution and dilutions thereof were inoculated into the different test media. All media were incubated at 55° C. Of the nine soil samples eight contained the "hard swell" type, six contained *Cl. nigrificans* and none contained the "flat sour" type of thermophiles. Both samples of soil previously fertilized with pea vines contained the "hard swell" type and *Cl. nigrificans* in appreciable numbers, but none of the "flat sour" type. Both samples of cured manure carried the two former types in large numbers, whereas only occasional spores of the two types were found in the fresh material.

Although the numbers of samples examined are too small to justify the drawing of general conclusions, the foregoing results confirm the isolation of *Cl. nigrificans* and the "hard swell" type from manure and indicate their abundance in fertilized soil. The enormous increase in numbers of these types during the curing of manure may be significant in the infection of fields through fertilization.

In a further study of the question of the foci of infection of these organisms in cannery machinery, the results of tests made in another canning factory during the pea packing season should be mentioned. The canned products put out at this place likewise had not shown excessive spoilage for many years. Contrary to expectations, however, large numbers of the "flat sour" type of thermophile were found at several points in the system, and in the finished product. The results of the examination of samples of peas as they underwent the various treatments necessary before processing showed a total absence of the typical "flat sour" type in the fresh product, but an average of 1,000 per gram on peas removed from an accessory blanching tank. As all peas passed through the same filling machines, a large part of the entire output of this factory had become infected with "flat sour" thermophiles, and of 22 cans of the finished product incubated at 55° C., 19 showed "flat sour" spoilage, indicating that most of the season's pack still harbored these organisms in a viable state. An efficient cooling system, which insured the storing of the cans in a well-cooled condition, is all that prevented "flat sour" spoilage on a large scale.

SUMMARY

Studies of the distribution of spoilage thermophiles have shown an abundance of *Cl. nigrificans* and the "hard swell" type in soil and in stable fertilizer. Curing of the manure is believed to increase their number markedly. The "flat sour" type was found only in sugar. Tests in canning establishments have shown that the absence of pronounced thermophilic spoilage of the canned product may or may not be a true indication of the absence of the spoilage thermophiles from canning systems.

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THE FREE ENERGY DECREASE OF ALCOHOLIC FERMENTATION

By ELLIS I. FULMER and EINAR LEIFSON.

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From the Laboratory of Biophysical Chemistry, Iowa State College.

The living cell is a special type of energy transformer which obtains its energy by catalyzing certain chemical reactions. It is a matter of importance then, to have a criterion for the amount of energy available to an organism from a given reaction. The criterion commonly used by the biologist has been the heat of reaction, obtained either by the use of heats of formation or heats of combustion of the substances involved. However, the physical chemist has for some time recognized the fact that the heat of reaction is not a criterion of the maximum available energy for the reaction, but that in its stead there must be employed the principle of maximum work or free energy. This issue has been concisely stated by Taylor (1924) to the effect that "The heat of reaction is not the true thermodynamic criterion for chemical reaction. On the contrary, the change of free energy, the capacity of the system to do chemical, electrical or mechanical work will be shown to be the correct measure of the driving force of a reaction. In certain special, but accidental cases the heat of reaction and the free energy of the process may be equal. In the majority of cases this will not be true."

The free energy concept has been applied quantitatively to microbiological reactions only recently, especially by Linhart (1920), Baas-Becking and Parks (1927), Burk (1927) and Buchanan and Fulmer (1928). However, it seemed advisable that there be a summary of the working tools for such calculations and their detailed application to a specific reaction of interest in biology. It is obvious that the discussion in this paper is in no way a substitute for a thorough study of the subject of thermodynamics. Its purpose is merely to point out in some detail typical methods and sources of information for further application.

It is the purpose of this essay, then, to outline very briefly the standard physico-chemical formulations underlying the calculation of the free energy decrease and their application to a specific microbiological process, i. e. to alcoholic fermentation.

This discussion will be taken up in the following order:

- I. The available energy of a reaction.
 1. Introduction
 2. At constant volume
 3. At constant pressure
 4. Summary.
- II. The calculation of the free energy decrease of a reaction.
 1. Standard concentrations (standard state)
 2. Constant concentrations not standard.

III. The free energy decrease of alcoholic fermentation.

1. Introduction.
2. Free energy of formation of CO_2 at various pressures.
3. The free energy decrease of formation of ethyl alcohol at various concentrations.
4. The free energy decrease of formation of sucrose solutions.
 - (a) From freezing point data
 - (b) From vapor pressure data
 - (c) The effect of alcohol on the free energy of dilution of sucrose.
5. The free energy decrease of the reaction, $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O} = 4\text{CO}_2 + 4\text{C}_2\text{H}_5\text{OH}$ under various conditions as to concentrations of the reacting substances.

IV. General discussion and significance of the free energy calculations for the fermentation reaction.

V. Summary.

I. The Available Energy of a Chemical Reaction.

1. INTRODUCTION.

The maximum work which a reacting system is capable of doing, that is the available energy, may be treated under two headings, namely, the reaction takes place at constant volume or it takes place at constant pressure. In the first instance the pressure (P) and in the latter the volume (V) is a variable. The fundamental relationships for the above involve quantities symbolized by A and F respectively. Since these two functions have in several instances been confused a brief review is presented of these quantities and their inter-relationships. Also there will be pointed out the relation between these functions and the heat of the reaction.

2. THE REACTION TAKES PLACE AT CONSTANT VOLUME.

The first law of thermodynamics, a statement of the law of conservation of energy, may be formulated as follows:

$$(1) A + Q = U$$

in which

A = The work content or available energy.

Q = The non-available, "latent", or "bound" energy.

U = The total or intrinsic energy.

The above simply states the fact that of the total energy content of a system (U) a portion may not be available for work, but that such total energy may be divided into that which is available (A) and that not so available or bound (Q)

also,

$$(2) A = U - Q$$

that is, the available or utilizable energy for performing work is the difference between the total energy and that which is bound.

Now assume that two systems react, then,

$$(3) \quad \Delta A = \Delta U - \Delta Q$$

in which

ΔA = The increase in available energy

ΔU = The increase in intrinsic or total energy

ΔQ = The gain in heat from the surroundings, i. e. the heat absorbed.

That is, the gain in free energy is the difference between the increase in total energy and the heat absorbed. When external work is done ΔA is negative. If the work done is designated by ΔW , as is often the case, then $\Delta W = -\Delta A$.

Assume that the reaction takes place in such a manner that no work is done by or on the system, i. e. $\Delta A = 0$, and there is no volume change, then, all the change in intrinsic energy will appear as heat, i. e. $-\Delta U = -(\Delta Q)_v$ = the heat of the reaction, or,

$$(4) \quad \Delta A = (\Delta Q)_v - (\Delta Q)$$

The quantity $(\Delta Q)_v$ is a constant for a given set of conditions while ΔA and ΔQ may vary. The quantity $(\Delta Q)_v$ must not be confused with (ΔQ) . This latter quantity by definition is the sum of the gain in available energy and the heat of reaction.

The above relations deal with the first law of thermodynamics and give no clue as to the direction in which the reaction proceeds. The second law of thermodynamics is a formal statement against the production of a perpetual motion machine. In the words of Clausius "It is impossible for a self-acting machine working in a cycle to convey heat from a body at a low temperature to one at a higher temperature; or heat cannot of itself (i. e. without the performance of work by some external agency) pass from a cold to a less cold body."

This is the law of dissipation of energy which means that in practice not all of the intrinsic energy of a system can be converted into external work but that during the reaction some of the energy is "lost" as heat.

This law leads to the generalization that if no external work is done and the volume is constant, that,

$$(5) \quad \Delta S = \frac{\Delta Q}{T} = \frac{\Delta U}{T}$$

in which ΔS is the entropy increase.

Substituting the value of $T\Delta S$ in Eq (3) we obtain

$$(6) \quad \Delta A = \Delta U - T\Delta S$$

and in Eq (4)

$$(7) \quad \Delta A = (\Delta Q)_v - T\Delta S$$

The third law of thermodynamics states that at absolute zero, i. e. $T = 0$, $Q = 0$ and the entropy of pure solids or liquids is zero, or,

$$(8) \quad S = \frac{Q}{T}$$

Substituting the value of TS for Q in (2) we obtain,

$$(9) \quad A = U - TS$$

The differentiation of equation (9) gives

$$(10) \quad dA = dU - TdS - SdT$$

but according to Eq (5) $TdS = dU$ and,

$$(11) \quad dA = dU - dU - SdT$$

and,

$$(12) \quad -S = \left(\frac{\delta A}{\delta T} \right)_v$$

Substituting (12) in (9), we obtain,

$$(13) \quad A = U + T \left(\frac{\delta A}{\delta T} \right)_v$$

and for a system reacting reversibly and isothermally without doing external work,

$$(14) \quad \Delta A = (\Delta Q)_v + T \left(\frac{\delta(\Delta A)}{\delta T} \right)_v$$

Equation (14) shows that the available energy (maximum work) $(-\Delta A)$ of a reaction and the heat of reaction $(-\Delta Q)_v$ at constant volume are identical only when the available energy does not change with

temperature, i. e., when $\left(\frac{\delta(\Delta A)}{\delta T} \right)_v = 0$.

If $\left(\frac{\delta(\Delta A)}{\delta T} \right)_v$ is negative, $-\Delta A > -(\Delta Q)_v$

If $\left(\frac{\delta(\Delta A)}{\delta T} \right)_v$ is positive, $-\Delta A < -(\Delta Q)_v$

That is, values for the heat of reaction at constant volume cannot be assumed to be identical with the available or free energy of a reaction taking place at constant volume.

3. THE REACTION TAKES PLACE AT CONSTANT PRESSURE.

In the above development constant volume was assumed. If the volume is not constant the value of A (equation 2) becomes $A + PV$ or,

$$(15) \quad F = A + PV = U - Q + PV$$

in which F is called the free energy content and is defined as

$$(16) \quad F = A + PV$$

also,

$$(17) \quad F = U + PV - TS \text{ (from equation 4)}$$

The term $U + PV$ represents the heat content, H , hence the usual formulation,

$$(18) \quad F = H - TS$$

This is the fundamental equation given by Lewis and Randall (p. 155, 1923).

Also,

$$(19) \quad \Delta F = \Delta H - T\Delta S$$

for a reaction taking place reversibly and isothermally at constant pressure, hence $-\Delta H$ is the heat of reaction at constant pressure (ΔH is defined as heat absorbed in the reaction), moreover,

$$(20) \quad \Delta F = \Delta U + T\Delta S + P\Delta V = (\Delta Q)_v - T\Delta S + P\Delta V$$

It is obvious that the heats of reaction at constant pressure and constant volume are equal only when the volume change is very small; under these conditions $\Delta F = \Delta A$. Differentiation of equation (17) gives,

$$(21) \quad dF = dU - SdT - TdS + PdV + VdP$$

The value of entropy,

$$(22) \quad S = \frac{Q}{T} = \frac{U + PV}{T} \text{ (If no external work is done)}$$

hence,

$$(23) \quad dS = \frac{dU + PdV + VdP}{T}$$

$$(23a) \quad TdS = dU + PdV \text{ since } P = \text{constant}$$

Substituting the value of TdS in equation (21)

$$(24) \quad dF = dU - dU - PdV - SdT + PdV + VdP$$

and,

$$(25) \quad dF = -SdT + VdP$$

for at constant pressure, $dP = 0$

$$(26) \quad \left(\frac{\delta F}{\delta T} \right)_P = -S \text{ (Compare with equation 12)}$$

Substituting in (18)

$$(27) \quad F = H + T \left(\frac{\delta F}{\delta T} \right)_P$$

and for a system reacting isothermally and reversibly,

$$(28) \quad \Delta F = \Delta H + T \left(\frac{\delta(\Delta F)}{\delta T} \right)_P \text{ a relation similar to equation (14)}$$

The free energy decrease, $(-\Delta F)$, of the reaction is equal to the heat of the reaction, $(-\Delta H)$, only when the free energy decrease is independent of the temperature, i. e., $\left(\frac{\delta(\Delta F)}{\delta T} \right)_P = 0$,

when,

$$\left(\frac{\delta(\Delta F)}{\delta T} \right)_P \text{ is positive, } -\Delta F < -\Delta H$$

and

$$\left(\frac{\delta(\Delta F)}{\delta T} \right)_P \text{ is negative, } -\Delta F > -\Delta H$$

4. IN SUMMARY, heats of reaction at constant volume or at constant pressure are not the true measure of the available energy under the specified conditions, instead the functions ΔA or ΔF should be used for reaction at constant volume or constant pressure respectively. These two functions are identical only for small volume changes.

The appended list of equations involving the functions A and F arranged in parallel should be useful in summarizing their interrelationships.

TABLE I

Summary of relations involving A and F .

A	F
Constant volume	Constant pressure
$A = U - Q$ (1)	$F = A + PV = U - Q + PV$ (15)
$A = U - TS$ (9)	$F = U + PV - TS = H - TS$ (17) (18)
$\Delta A = \Delta U - T\Delta S$ (6)	$\Delta F = \Delta U - T\Delta S + P\Delta V$ (20)
$\Delta A = (\Delta Q)_V - T\Delta S$ (7)	$\Delta F = (\Delta Q)_V - T\Delta S - P\Delta V$ (20)
$S = \frac{Q}{T}$ (8)	$S = \frac{U + PV}{T}$ (22)
$A = U - TS$ (9)	$F = U + PV - TS = H - TS$ (17) (18)
$dA = dU - TdS - SdT$ (10)	$dF = dU - SdT - TdS + PdV + VdP$ (21)

$$-S = \left(\frac{\delta A}{\delta T} \right)_V \quad (12)$$

$$-S = \left(\frac{\delta F}{\delta T} \right)_P \quad (26)$$

$$A = U + T \left(\frac{\delta A}{\delta T} \right)_V \quad (13)$$

$$F = H + T \left(\frac{\delta F}{\delta T} \right)_P \quad (27)$$

$$A = (\Delta Q)_V + T \left(\frac{\delta(\Delta A)}{\delta T} \right)_V \quad (14) \quad \Delta F = \Delta H + T \left(\frac{\delta(\Delta F)}{\delta T} \right)_P \quad (28)$$

Also, it should be noted that there is much disagreement in the literature with regard to the symbols used for the various thermodynamic functions. In Table II will be found the summary of various important symbologies according to Eucken, Jette and La Mer (1925).

TABLE II
Symbols used in thermodynamics.

Authority								
Function	Condi- tions of restraint	Gibbs	Planck	W. McC Lewis	MacDou- gal	G. N. Lewis	Eucken (1)	Eucken (2)
$U-TS$	T, V	Ψ	F	f	F	A	$-A_T$	$-A_{T,V}$
$U-TS+PV$	T, P	ζ	βT	Φ	Φ	F'	$-A_m$	$-A_{T,P}$
$U+PV$	T, P	K	—	—	H	H	W	H
$(U-TS+PV)$ m	T, P	μ	—	$\delta\Phi$ δm	$\delta\Phi$ δm	F'	—	$-A_{T,P}$

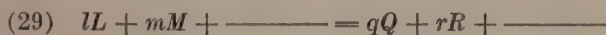
(1) 2nd German edition.

(2) Translation by Jette and La Mer.

II. The Calculation of Free Energy Decrease of Reaction.

1. THE STANDARD STATE.

At a given temperature the value of the free energy decrease ($-\Delta F$) of a reaction depends upon the concentrations of the reacting materials. It is necessary, therefore, to formulate a "standard state" which will be most convenient for general use. The relations involved between ΔF and "activities" of the reacting materials may be arrived at by the consideration of the reaction,



for which,

$$(30) \quad \Delta F - \Delta F^\circ = RT \ln \frac{\left(a_Q^q \right) \times \left(a_R^r \right)}{\left(a_L^l \right) \times \left(a_M^m \right)} - RT \ln \frac{\left(a_Q^\circ{}^q \right) \times \left(a_R^\circ{}^r \right)}{\left(a_L^\circ{}^l \right) \times \left(a_M^\circ{}^m \right)}$$

in which ΔF and ΔF° represent the free energy of the reaction at the two activities a and a° . If the "standard state" be defined as that in which the activity of each reacting substance is unity, equation (30) becomes,

$$(31) \quad \Delta F - \Delta F^\circ = RT \ln \frac{\left(a \begin{smallmatrix} q \\ Q \end{smallmatrix}\right) \times \left(a \begin{smallmatrix} r \\ R \end{smallmatrix}\right)}{\left(a \begin{smallmatrix} l \\ L \end{smallmatrix}\right) \times \left(a \begin{smallmatrix} m \\ M \end{smallmatrix}\right)}$$

In dilute solutions concentrations may be substituted for activities. Under these conditions the symbol ΔF° represents the free energy of the reaction in the standard concentration (one molal) or at the standard pressure (one atmosphere). If activities need to be employed the standard state is at one molal activity and at a fugacity of one atmosphere. The standard temperature is taken as 25° C. or 298° A. For example, the value for ΔF°_{298} for NO_2 is 27 Cal., i. e., the free energy decrease of formation per mol of nitrite ion in molal concentration (activity) at 25° is 27 Cal. The free energy decrease of formation of a compound in the standard state is designated by $-\Delta F^\circ_{298}$ and represents the free energy decrease of formation per mol of substance in the particular state prevailing at 25° C.

The value for ΔF for a gas at any fugacity, f_2 , may be calculated from the value at any other fugacity, f_1 , by the following relation,

$$(33) \quad \Delta F_{f_2} = \Delta F_{f_1} + RT \ln \frac{f_2}{f_1}$$

If the standard be taken as one atmosphere fugacity, then, in large Calories,

$$(34) \quad \Delta F_{298} = \Delta F^\circ_{298} + \frac{RT}{1000} \ln f_2 = \Delta F^\circ_{298} + 1.365 \log f_2$$

For ordinary pressure ranges, as will be shown later, pressures may be substituted for fugacities. For a volatile solute, as ethyl alcohol, the pressures used will be the partial vapor pressures. For example, if ΔF°_{298} represents the free energy increase of formation per mol of alcohol in molal concentration (activity) the value substituted in the standard equations will be the partial vapor pressure of the alcohol at any other molality.

For non-volatile solutes the following relationship holds,

$$(35) \quad \Delta F_{298(a_1)} = \Delta F^\circ_{298} + 1.365 \log a_2$$

in which ΔF°_{298} represents the free energy of formation per mol at molal activity. This value may be calculated as follows. The free energy of dilution is,

$$(36) \quad \Delta F = \frac{-RT}{1000} \ln \frac{a_s}{a} = -1.365 \log \frac{a_s}{a}$$

in which a_s is the activity of the solute in saturated solution. This will have the same value of ΔF as for the pure substance since in a saturated

solution the solid is in equilibrium with the solute. If a be taken as "standard", that is $a = 1$, then,

$$(37) \quad \Delta F_{298}^{\circ} = -1.365 \log a_s$$

While the concentrations may be substituted for activities in equation (35) for usual ranges in biological media this cannot be safely done in equation (37) for in a saturated solution the activity may vary greatly from concentration. This will be illustrated later in the instance of solutions of sucrose.

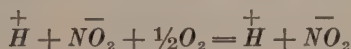
In Table III are given values of $1.365 \log a$ for various values of a .

TABLE III

Values of $1.365 \log a$ for Various Values of a .

a	$1.365 \log a$
2	+0.411
1	0
0.5	-0.411
0.2	-0.955
0.1	-1.365
0.01	-2.730
10^{-3}	-4.095
10^{-4}	-5.460
10^{-5}	-6.825
10^{-6}	-8.19
10^{-7}	-9.55

The use of these relationships may be illustrated by the reaction,

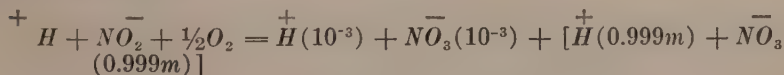


$$0 - 27 - 0 = -0 - 50 + x$$

$$- \Delta F_{298}^{\circ} = 23 \text{ Cal.}$$

that is, the free energy decrease of the reaction per mol of nitric acid formed in molal concentration of nitric acid and of nitrous acid is 27 Cal. It must be noted that the assumption is made that all reacting materials are constantly at molal concentration (activity).

Now assume that the nitric acid is removed from solution in such a manner that its concentration (activity) is constantly 10^{-3} that of the nitrous acid, then,



$$0 - 27 - 0 = -4.1 - 50 - 4.1 + x$$

$$- \Delta F_{298}^{\circ} = 31.1 \text{ Cal.}$$

This value is considerably higher than that previously obtained by assuming that the concentrations of the reactants and resultants were

equal. In the above equation the quantities in the brackets represent the concentrations of resultants removed to cause the constant concentration of 10^{-3} .

Again, assume that the concentration of the HNO_2 is 10^{-3} and the HNO_3 constant at 1, then,

$$\begin{aligned} & [\overset{+}{H}(0.999) + \bar{N}\bar{O}_2(0.999)] + \overset{+}{H}(10^{-3}) + \bar{N}\bar{O}_2(10^{-3}) + \frac{1}{2}O_2 = \overset{+}{H} \\ & \quad + \bar{N}\bar{O}_3 \\ & - \Delta F_{298} = 14.8 - 4.1 - 31.1 = 0 - 50 + x \end{aligned}$$

It is obvious from the above numerical examples that the free energy decrease, i. e., the available energy, of the reaction, increases when the concentration of resultants becomes relatively less than that of the reactants and vice versa. If the concentration of HNO_3 were 10^9 that of the HNO_2 the reaction would yield no energy, in fact the value of $-\Delta F_{298} = -1.57$. The oxygen pressure will also have an influence. In the above example it was assumed to be at one atmosphere pressure (fugacity). If the relative concentration of the nitrous and nitric acid did not change, for each tenfold increase in the fugacity of the oxygen the value of $-\Delta F$ will increase 1.36 large calories and will decrease a like amount for each tenfold decrease in pressure.

TABLE IV

Free Energy Decrease of Formation and Heat of Formation of Several Compounds.

[Parks and Huffman (1926); Parks and Kelley (1925); Parks and Anderson (1926); Parks (1925); Lewis and Randall (1923), and various sources.]

Compound	$-\Delta F_{298}$	$-\Delta H$
Methyl alcohol	44.50	61.7
Ethyl alcohol	44.00	69.9
n-Propyl alcohol	44.10	73.8
iso-Propyl alcohol	47.70	80.5
n-Butyl alcohol	44.10	82.8
tert.-Butyl alcohol	49.90	88.4
Ethylene glycol	82.50	113.4
Glycerol	116.70	161.7
Mannitol	226.2	314.9
Dulcitol	228.1	342.9
Glucose	219.1	302.6
Erythritol	152.9	214.8
Formic acid	84.04	102.6
Acetic acid	96.60	117.0
n-Butyric acid	92.5	125.3
Palmitic acid	89.00	214.4
Oxalic acid	167.5	197.6
Acetone	38.00	66.3
Ethyl ether	36.60	70.5
Carbon monoxide (graphite)	32.51	26.1
Carbon dioxide (graphite)	94.26	94.25
Urea	47.28	80.8
Water	56.56	69.0
Formaldehyde	33.0	42.5
*Sucrose	380.0	535.6

*This value was kindly furnished in a private communication by G. S. Parks, who states that the value "is probably good to $\pm 1\%$."

The principles outlined above have only recently been employed in the analysis of the available energy of biological processes. One of the early attempts to compare free energy decrease and heats of combustion was made by Baron and Polanyi (1913). These authors studied the oxidation of glucose, of albumin and the transformation of glucose into fats, and found that in general the free energy decrease was from 5-13% greater than the heat of reaction. They made use of an approximate formula of Nernst and employed the chemical constants calculated by this author. Moreover, they employed the heat of formation of water in the liquid state when in fact the reaction involved the formation of water vapor, that is, they neglected the $P\Delta V$ factor. Simon (1922) made more accurate calculations, but still used the Nernst constants which are now considered to be unreliable. The relations developed by Lewis and co-workers have recently been applied to the energy relationships of the autotrophic bacteria by Baas-Becking (1927) and by Linhart (1920) and Burk (1927) to nitrogen fixation.

While these later workers employed concentrations other than standard, in each case they assumed constant concentrations of reacting materials throughout the reaction. In the analysis of the free energy changes in alcoholic fermentation to be outlined, account is taken of the fact of changing concentrations, resultants appearing at the expense of the reactants.

In Table IV are given values for the free energy decrease and heats of formation of several compounds of special interest in bacteriological reactions.

It is apparent that the values for $-\Delta F_{298}$ may differ widely from those for $-\Delta H$. Moreover, there is no appreciable change in the values of $-\Delta F$ in a homologous series while the value of $-\Delta H$ increases regularly with the introduction of the CH_2 group. It follows that the values for $-\Delta F$ for a given reaction, involving members of a homologous series, will increase more rapidly than the values for $-\Delta H$ in ascending the series. Parks and Kelley (1925) generalize further by stating that the substitution of an OH group for a hydrogen leads to a free energy decrease of about 35.5 Cal.

III. The Free Energy Decrease in Alcoholic Fermentation.

1. INTRODUCTION.

In the following development we shall assume that sucrose yields only ethyl alcohol and carbon dioxide as represented by the equation,



During the course of the reaction the concentration of the reactant is decreasing while the concentrations of the resultants are increasing. These changes all lead to a decrease of available energy for the reaction. It is obvious then that not only cannot standard states be used for calculating the free energy decrease for the reaction, but that variation in concentrations must be taken into consideration. Other factors further complicate the situation; for example, the effect of the salts in the medium, of metabolic products, change of pH and so on, upon the activities of the reacting

materials. Only one of these factors, the effect of the alcohol upon the solubility, and hence upon the activity of the sucrose, will be considered.

2. THE FREE ENERGY OF FORMATION OF CO_2 AT VARIOUS PRESSURES.

The fugacity of a gas may be calculated for a given pressure by means of the following relation,

$$(39) \quad \log f = \log P - \frac{1}{2.303RT} \int_0^P a dP$$

in which a represents the difference between the ideal volume (the volume had it obeyed the gas laws) and the actual volume at the given pressure. Values of a were calculated from data given by Landolt-Börnstein. The area under the curve obtained by plotting a against P , from 0 to any value

of pressure, P , gives the value of the expression $\int_0^P a dP$

In Table V are given values of the fugacity of CO_2 at various pressures as calculated by means of equation (39).

TABLE V
Values for Fugacity of CO_2 .

P atmospheres	$\log P$	$\int_0^P a dP$	$\frac{1}{2.303RT} \int_0^P a dP$	$\log f$	f
1	0	0	0	0	1.
5	0.699	525	0.00915	0.6898	4.896
10	1.000	1,050	0.0183	0.9817	9.587
20	1.301	2,115	0.0369	1.264	18.37
30	1.477	3,190	0.0557	1.421	26.39
40	1.602	4,270	0.0745	1.528	33.7
50	1.699	5,400	0.0942	1.605	40.25
100	2	15,450	0.270	1.730	53.71
200	2.301	26,850	0.468	1.833	68.08
400	2.602	33,700	0.588	2.014	103.3
600	2.778	34,350	0.598	2.180	151.4
800	2.903	32,400	0.567	2.337	217.3

The values for the free energy decrease of formation of CO_2 at various values of fugacity may be calculated by means of equation (34) in which the value of $-\Delta F^\circ_{298}$ is taken as 94.26 Cal. Values of $-\Delta F$ for carbon dioxide at various pressures are found in Table VI.

TABLE VI
Values for $-\Delta F$ for CO_2 at Several Pressures.

P (atm.)	$-\Delta F$ (Cal.)	P (atm.)	$-\Delta F$ (Cal.)
10^{-10}	108.1	20	92.51
10^{-8}	105.4	30	92.29
10^{-6}	102.6	40	92.14
10^{-4}	99.81	50	92.04
10^{-3}	97.04	100	91.86
10^{-1}	95.64	200	91.72
1	94.26	400	91.47
5	93.31	600	91.24
10	92.92	800	91.02

It will be noted that up to 50 atmospheres pressure the use of pressure instead of fugacity makes little difference in the calculated values for $-\Delta F$. It is obvious that any method for removal of the CO_2 during fermentation will increase the available energy and that keeping the CO_2 under pressure will cause the reverse.

3. THE FREE ENERGY DECREASE OF FORMATION OF ETHYL ALCOHOL AT VARIOUS CONCENTRATIONS.

The free energy decrease of formation of ethyl alcohol may be calculated by means of equation (33). Since the alcohol does not attain a high concentration during fermentation the partial vapor pressure may be substituted for the fugacity. Values so calculated from data by Wrewsky (1913) are given in Table VII.

TABLE VII
Free Energy Decrease of Formation of Ethyl Alcohol.

Molal concentration	% alcohol by weight	Molar concent.	Partial P (alcohol)	P/P_0	$\frac{(\Delta F^\circ - \Delta F')}{2.303RT \log P/P_0}$	$-\Delta F$
	100		129.8	1	0	45.100
4.94	22.0	4.63	44.6	0.342	-0.636	45.736
3.47	15.9	3.37	37.1	0.286	-0.740	45.840
2.21	10.0	2.16	29.0	0.223	-0.890	45.990
1.10	5.0	1.08	18.0	0.138	-1.170	46.270
0.57	3.00	0.55	12.0	0.092	-1.420	46.520
0.21	1.00	0.21	5.0	0.038	-1.940	47.040
0.21×10^{-1}	10^{-1}	0.21×10^{-1}	0.5	3.8×10^{-3}	-3.300	48.400
0.21×10^{-2}	10^{-2}	0.21×10^{-2}	0.5×10^{-1}	3.8×10^{-4}	-4.670	49.770
0.21×10^{-3}	10^{-3}	0.21×10^{-3}	0.5×10^{-2}	3.8×10^{-5}	-6.035	51.135
0.21×10^{-4}	10^{-4}	0.21×10^{-4}	0.5×10^{-3}	3.8×10^{-6}	-7.400	52.500
0.21×10^{-5}	10^{-5}	0.21×10^{-5}	0.5×10^{-4}	3.8×10^{-7}	-8.765	53.865
0.21×10^{-10}	10^{-10}	0.21×10^{-10}	0.5×10^{-9}	3.8×10^{-13}	-15.590	60.690

4. THE FREE ENERGY DECREASE OF FORMATION OF SUCROSE SOLUTIONS.

In order to use equation (34) for the free energy of formation of sucrose it will be necessary to calculate the activity of sucrose solutions up to saturation. It was necessary to use three sets of data for these calculations since it was found that the value of a_2 varied considerably with the temperature.

(a) Calculations from Freezing Point Data.

For these calculations the following relationship was used (Lewis and Randall p. 286),

$$(40) \quad \ln \frac{a_2}{m} = \int_0^m j d \ln m - j + \int_0^m 0.00057 \frac{\Theta}{m} d\Theta$$

in which Θ is the freezing point lowering of molal concentration at activity a_2 and,

$$(41) \quad j = 1 - \frac{\Theta}{\lambda m}$$

in which $\frac{\Theta}{\lambda m}$ is the ratio between the given molal lowering of freezing point and λm the molal lowering at infinite dilution, i. e., assuming that the gas law holds.

The value of the first integral (equation 40) which we shall designate as (A) is the area under the curve from 0- m obtained by plotting $\frac{-j}{m}$ against m . The value of the second integral (B) is the area from 0 - m under the curve of $0.00057 \frac{\Theta}{m}$ against Θ .

In order to save space the complete calculations are not given. The activity of the sucrose in saturated solution (5 molal) at -15° was found to be 15, i. e., a_s at $-15^\circ = 15$.

(b) *From Vapor Pressure Data.*

The calculations from vapor pressure data may be made by use of the following equation (Lewis and Randall),

$$(42) \quad \ln \frac{a_2}{m} = -h - \int_0^m \frac{h}{m} dm$$

in which $h = \frac{55.51 \ln a_1}{m} + 1$

$$\text{and } a_1 = \frac{P}{P_0}$$

where P_0 is the vapor pressure of pure water and P that of the solvent in the

solution at the given temperature. The value of the integral $\int_0^m \frac{h}{m} dm$

is the value from 0 - m of the area under the curve obtained by plotting $\frac{h}{m}$

against m . The value for a_s so calculated was 15.4 at 5.25 molal at 0° , and 19.5 (6.11 molal) at 30° . From these data the value of a_s at 25° was taken to be 18.0. Using this value in equation (34) values for the free energy decrease of dilution were calculated and given in Table VIII, Column 4.

(c) *The Effect of Alcohol Upon the Free Energy of Dilution of Sucrose.*

For a complete analysis of the free energy changes during the reaction account should be taken of the effect of changing concentrations upon the solubility (hence the activities) of the reacting substances. This may be illustrated by the effect of the increase in concentration of the alcohol

TABLE VIII
Free Energy of Dilution of Sucrose.

Molal concentration	$\frac{a_2}{a_s}$	$\text{Log } \frac{a_2}{a_s}$	ΔF , 0% alcohol	ΔF , 20% ⁽¹⁾ alcohol	ΔF , 50% ⁽¹⁾ alcohol
0.001	0.000055	-4.255	-5.810		
0.01	0.00055	-3.255	-4.445		
0.1	0.0055	-2.255	-3.080	-3.02	2.40
0.995	0.066	-1.18	-1.610	-1.41	0.960
1.65	0.124	-0.906	-1.238	-1.04	0.560
2.37	0.206	-0.686	-0.936	-0.720	2.240
3.28	0.337	-0.472	-0.644	-0.440	+0.040
4.12	0.494	-0.306	-0.418	-0.220	+0.280
5.35	0.785	-0.105	-0.143	+0.040	+0.560

⁽¹⁾These data were not calculated by means of values in columns 2 and 3 but from values of a_2/a_s for 20% and 50% alcohol.

upon the activity of the sucrose. The activity of the sucrose in any concentration of alcohol is,

$$(43) \quad a_2^1 = \frac{\text{solubility in water}}{\text{Solubility in the solution}} \times a_2$$

The effect of 20% and 50% alcohol upon the free-energy decrease of solution of sucrose is shown in Columns 5 and 6, Table VIII. It is evident that concentrations of alcohol produced during an ordinary fermentation will not cause enough variation in these values to cause serious error in using the values for pure water.

5. THE FREE ENERGY OF THE FERMENTATION REACTION.

From data given in the preceding tables it is possible to calculate the free energy decrease of the reaction,



in any concentration of sucrose for,

$$(43) \quad - \left(\frac{\Delta F_{\text{sugar}}^{\circ}}{298} + \frac{\Delta F_{\text{sugar}}}{298} \right) + \left(\frac{4\Delta F_{\text{alcohol}}^{\circ}}{298} + \frac{4\Delta F_{\text{alcohol}}}{298} \right) + \left(\frac{4\Delta F_{\text{CO}_2}^{\circ}}{298} + \frac{4\Delta F_{\text{CO}_2}}{298} \right) = \Delta F_{\text{reaction}}$$

and,

$$(44) \quad \left(-\frac{\Delta F_{\text{sugar}}^{\circ}}{298} + \frac{4\Delta F_{\text{alcohol}}^{\circ}}{298} + \frac{4\Delta F_{\text{CO}_2}^{\circ}}{298} \right) - \left(\frac{\Delta F_{\text{sugar}}}{298} + \frac{4\Delta F_{\text{alcohol}}}{298} + \frac{4\Delta F_{\text{CO}_2}}{298} \right) = \Delta F_{\text{reaction}}$$

Since the problem involves the change in free energy of reactions per mol of an initial concentration (M_1) to a final concentration, (M_2), we may write

$$(45) \quad 4\Delta F + 4 \underset{\text{alcohol}}{\Delta F} - \underset{\text{CO}_2}{\Delta F} = (\Delta F - K) \underset{\text{sugar}}{\Delta F}$$

The expression $(\Delta F - K)$ will give the desired information. Theoretically, at the start with zero concentration of CO_2 and of ethyl alcohol, it is obvious that the free energy decrease of formation at these concentrations approaches infinity. For purposes of simplification we shall assume that the inoculation carries a given small concentration of alcohol. It is also assumed that none of the alcohol leaves the solution. We shall also assume that the medium was originally saturated with CO_2 at one atmosphere of air and that the concentration is constant at that figure, i. e., the partial pressure does not change. This simply assumes free contact with the atmosphere so that mixing is complete. Normally at an atmosphere of air the partial pressure of the CO_2 is 0.003 atmosphere. Using equation (34) the value of ΔF is -4.800 Cal. and $4\Delta F = -19.200$ Cal. We may now write,

$$(46) \quad (\Delta F - K) = 4\Delta F - \underset{\text{alcohol}}{\Delta F} - 19.20. \underset{\text{sugar}}{\Delta F}$$

In Table IX are found values of $(\Delta F - K)$ calculated for four initial concentrations of sucrose and three initial concentrations of ethyl alcohol. These data were calculated by means of equation (46).

The meaning of the figures in Table IX may be illustrated by the data in the second column. Assume one molal concentration of sucrose in the presence of 0.21×10^{-3} molal ethyl alcohol, and that CO_2 concentration is constant as previously indicated. The value of $(\Delta F - K)$ under these conditions is -41.74 , that is, the free energy decrease per mol of sugar

TABLE IX.

Values of $(\Delta F - K)$ for Various Concentrations of Sucrose and Alcohol.

Initial EtOH <i>M</i>	0.21×10^{-3}				0.21×10^{-3}				0.21			
% by Wt.	0.001				0.1				1%			
Sucrose Initial Molal Con- centration	$-(\Delta F - K)$				$-(\Delta F - K)$				$-(\Delta F - K)$			
1.00	41.74				30.80				25.36			
0.90	23.67				23.63				23.03			
0.80	22.52				22.48				22.16			
0.70	21.74	41.40			21.70	30.52			21.54	24.98		
0.60	21.16	23.38			21.12	23.24			21.00	22.64		
0.50	20.60	20.08	41.06		20.10	20.04	30.22		20.54	21.72	24.68	
0.45	-----	24.74			-----	24.70			-----	23.02		
0.40	20.21	21.25	22.93		20.17	21.21	22.89		20.05	21.11	22.29	
0.35	-----	22.23			-----	22.19			-----	21.75		
0.30	19.72	20.60	21.68	40.66	19.20	20.56	21.64	29.82	19.68	20.44	21.32	24.28
0.25	-----	21.20	24.32		-----	21.16	24.08		-----	20.88	22.60	
0.20	19.35	19.95	20.79	22.47	19.31	19.91	20.75	22.43	19.31	19.89	20.59	21.83
0.15	-----	20.34	21.62		-----	20.30	21.54		-----	20.10	21.14	
0.10	18.68	19.28	19.88	20.96	18.64	19.24	19.84	20.92	18.64	19.12	19.78	20.60
0.05	17.80	18.52	19.08	20.00	17.96	18.48	19.06	19.96	18.00	18.40	18.88	19.68
0.02	17.73	18.14	18.68	19.68	17.69	18.10	18.65	19.54	17.70	18.10	17.94	19.38
0.01	17.60	17.82	18.54	19.24	17.52	17.78	18.53	19.38	17.56	17.94	16.98	19.22

fermented with the above conditions constant is 41.74 Cal. greater than that calculated for standard states. Now assume an initial concentration of sucrose of $0.9M$ in the presence of the amount of alcohol in the solution when the molal concentration of the sucrose has changed from 1 to 0.90. The molality of the alcohol will be $0.4M + 0.21 \times 10^{-3}M$, or for all practical purposes $0.4M$. Under these conditions the value of $(\Delta F - K)$ is -23.7 Cal. The average value, then, of $(\Delta F - K)$ per mol of sucrose changed from $1M$ to $0.9M$ is the average of -41.74 and -23.67 or -32.70 . That is, the changing of concentration of the sucrose by fermentation from $1M$ to $0.9M$ has decreased the available energy per mol of sucrose fermented 18.1 Cal.

In Table X are given average values for $(\Delta F - K)$ per mol of sucrose fermented with various initial concentrations of alcohol for a change in molality, (ΔM) , of $0.3M$.

TABLE X.

Average Values of $(\Delta F - K)$ per mol of sucrose fermented when $\Delta M = 0.3$.

Initial Sucrose (M)	$0.21 \times 10^{-3}M$	$0.21 \times 10^{-3}M$	$0.21M$
1.00	-27.42	-24.65	-23.02
0.70	-26.52	-23.75	-22.61
0.50	-24.95	-23.36	-22.08

This means that the maximum difference between the free energy decrease of fermentation as calculated for standard states and for conditions likely to obtain in a fermenting mixture is about 27 Cal.

IV. General Discussion and Significance of the Free Energy Calculations for the Fermentation Reaction.

The free energy decrease of the fermentation reaction for components in the standard state may be calculated from data given in Table IV.

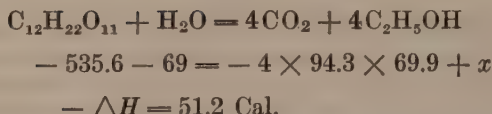


$$-380.0 - 56.6 = -4 \times 94.3 - 4 \times 44.0 + x$$

$$- \Delta F_{298}^{\circ} = 116.6 \text{ Cal.}$$

Referring again to Table X it is apparent that under the conditions specified the maximum free energy decrease for the conditions specified will be about 23% higher than that calculated for the standard state. Considering the probable inaccuracies in the values in free energy decrease of formation of the compounds as given in Table IV and the fact that the equation assumed for the fermentation does not represent accurately the products formed by biological action, the corrections from the standard state in this case are not of relatively great importance. However, it is worth while, in each instance, in the use of free energy changes of biological processes to analyze the situation along the lines presented above.

Finally it will be of interest to compare the value for the free energy decrease and for the heat of reaction for the fermentation. The heat of reaction is,



It is apparent that the free energy decrease of the reaction is two to three times the heat of reaction.

V. Summary.

A review has been presented of the relationships involved in the treatment of energy changes resulting from chemical reactions. The cases treated were those in which the reaction takes place at constant volume or at constant pressure. In the former instance the available energy, or the maximum work which the system is capable of doing, is designated by $-\Delta A$ and in the latter by $-\Delta F$. The fact was emphasized that only under special conditions is the heat of reaction identical with the free energy decrease of the reaction. Hence in dealing with the energy available to an organism from a given chemical change, whenever accurate data are available, the discussion should be based not upon the heat of reaction but upon the free energy function.

The free energy concept was applied to alcoholic fermentation assuming the quantitative relation:



Account was taken of the fact that the concentration of the reactant was continuously decreasing and that of the resultants increasing, both changes leading to a continuous decrease in the available energy of the reaction. In order to simplify the treatment it was assumed that the concentration of the carbon dioxide was constantly in equilibrium with the atmosphere and that there was present in the beginning of the experiment a definite low concentration of alcohol.

When all the above factors were taken into consideration it was found that the free energy decrease of the reaction under conditions likely to obtain in a normal fermentation was about 23% higher than that calculated on the assumption that all the materials were in the standard state. The free energy decrease is two to three times the value of the heat of the reaction.

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THE INHERITANCE OF RESISTANCE TO FOWL TYPHOID IN CHICKENS^{1, 2}

W. V. LAMBERT AND C. W. KNOX

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That inherent differences may play an important role in determining resistance to the bacterial diseases among animals has long been recognized, but not until quite recently have any systematic efforts been made to determine the exact part that these differences play. Many factors undoubtedly influence resistance to disease, including environment, general health, the degree of infection, virulence of the infecting organism and others; hence any study attempting to determine the role of inherent variability in resistance to disease must be carefully controlled.

Among the investigators who have reported upon the ability of selective breeding to increase resistance to disease, the results of Webster (1924-25) and of Roberts and Card (1926) are most outstanding. Webster found that the offspring of mice that had withstood an acute infection of mouse typhoid were much more resistant to infection of the same organism than were mice descended from parents that had never been subjected to an attack of this disease. In this work highly inbred lines of mice were used. Roberts and Card have observed a very similar situation in the resistance of chicks to bacillary white diarrhea.

Wright and Lewis (1921) observed marked differences in the resistance of inbred lines of guinea pigs to tuberculosis. One inbred line in particular gave a very high resistance, and when either males or females of this line were crossed with individuals from less resistant inbred lines, or with outside stock, a dominance of the resistance among the offspring was indicated. No apparent relationship between the factors of sex, age, weight at time of infection, rate of growth, and other things that might indicate general vigor was noted.

Hagedoorn, LaBrand and Hagedoorn (1920) have reported upon an inherited difference in resistance to a staphylococcus infection in mice. They attribute the difference in resistance to this infection, observed in albino mice, and susceptibility as observed in Japanese waltzers, as dependent upon one pair of genes.

PURPOSE

In this paper the writers are reporting the preliminary phases of an investigation begun with the purpose of attempting to determine whether or not it would be possible by selection to increase the resistance of chickens

¹Paper No. 20 from the Department of Genetics and No. 17 from the Department of Poultry Husbandry, Iowa State College, cooperating.

²The writers take this opportunity to express their gratitude to Professors R. E. Buchanan, C. H. Werkman, Charles Murray, G. W. Snedecor and A. E. Brandt for many valuable suggestions. Also to the Bacteriology Department for permission to use much of their equipment.

to fowl typhoid. The term resistance rather than immunity is used, for it is probable that no animal is completely immune to any disease. However, it seems probable that various degrees of resistance toward a given infection exist and if so it should be possible by selection to produce a strain of fowls having a high natural resistance.

METHODS

As foundation stock 219 White Leghorn chickens weighing between two and three pounds were secured and each bird was fed a massive dose of virulent fowl typhoid bacteria, *Shigella gallinarum* (Weldin 1926). Of this number 47.7 percent died (Lambert and Knox—In press). These tests were carried out over a three year interval. At the end of the three years this method of testing was discontinued and the plan of testing baby chicks for their resistance to fowl typhoid was adopted. A far greater number of chickens may be tested in the latter manner with the same equipment and at a much smaller cost, and in a study of this kind it is essential to have large numbers to make the results conclusive¹.

These birds were used as breeding stock and their offspring were subjected as six day old chicks to an inoculation of virulent typhoid bacteria. At the same time chicks of the same age that were hatched from non-tested parents were inoculated, and the chicks were then placed in brooders together. All were allowed an ample supply of feed and water and the conditions were kept as favorable as possible.

Each chick was inoculated intraperitoneally with 12,000,000 organisms suspended in physiological salt solution, a number that had been determined in previous trials to kill about 90 percent of all chicks infected, these chicks of course having as parents non-tested birds. The number of organisms injected was determined by use of the Helber counting chamber, and after counts on a given suspension had been made it was diluted to give the proper dosage. The organism used was grown on veal infusion agar slants for 20 hours at 37° C. In order to insure a uniform virulence the organism was reisolated every third week. Before using a reisolated organism it was always checked for its reaction on sugar media according to the method of May and Goodner (1926).

The Effect of Selection Upon Total Mortality

A total of 1305 chicks were tested with the fowl typhoid organism. The results of these tests are shown in Table I. Of these chicks 410 were descendants of parents that had both survived an acute infection of fowl typhoid. Of this group 168 or 40.9 percent died. Another group of 202 chicks that had as parents a typhoid-surviving male mated with non-tested females gave a mortality of 62.4 percent. Both of the above groups were Single Comb White Leghorns.

All other birds used were from parents not having been tested with the fowl typhoid organism. Of this group 405 were White Leghorns bred similarly to the ones above. Three hundred and fifty-nine or 88.6 percent of them died. A second group of 104 Rhode Island Reds showed a mortal-

¹Fowl typhoid is primarily a disease of nearly grown or adult birds, but if a highly resistant strain can be produced by selecting for resistance in the baby chicks, this strain can then be tested for their resistance as adults.

TABLE I. The mortality of chicks from parents that had survived an acute infection of fowl typhoid as contrasted with that of chicks whose parents had never been subjected to an attack of this disease.

Group	Parentage of chicks	Total No. Inoculated	Total No. ¹ dead	Total Pct. dead
I	Non-tested ♂ ♂ x ♀ ♀ (R. I. R.)	104	102	98.1
II	Non-tested ♂ ♂ x ♀ ♀ (W. P. R.)	80	66	82.5
III	Non-tested ♂ ♂ x ♀ ♀ (W. L. x R. I. R.)	104	89	85.6
IV	Non-tested ♂ ♂ x ♀ ♀ (W. L.)	405	359	88.6
V	Typhoid-surviving ♂ x non-tested ♀ ♀ (W. L.)	202	126	62.4
VI	Typhoid-surviving ♂ ♂ x ♀ ♀ (W. L.)	410	168	40.9

¹Mortality from the 3rd to 21st days, inclusive.

ity of 98.1 percent. A third lot of 104 chicks, descendants of White Leghorn males mated with Rhode Island Red females, gave a mortality of 85.6 percent. A group of 80 White Plymouth Rock chicks gave a mortality of 82.5 percent.

The difference between the two groups with typhoid-surviving ancestry, one with double and the other with single, is 21.5 percent. Between the group with double typhoid ancestry and all other White Leghorns from non-tested parents it is 47.7 percent, while between the lot with single typhoid ancestry and all other White Leghorns (non-typhoid-tested ancestry) it is 26.2 percent. All of these differences are very significant.

The probability that differences as great as the ones noted could be due to random sampling has been determined by the X^2 method of Fisher (1925). The value of X^2 for the respective differences is shown in Table II. From this table it will be seen that the odds against differences as great as those noted between the groups with typhoid ancestry and those with non-typhoid-surviving ancestry, being due to chance alone, are inconceivably great. It is also apparent from this table that some causes other than those of random sampling are operative in producing a difference as great as that noted between the lots with single and double typhoid-surviving ancestry.

TABLE II. Probability that differences as great as those noted between the respective groups are due to chance. The parentage of the groups compared is shown in Table I.

Groups compared	I & II	I & III	I & IV	II & IV	III & IV	IV & V	V & VI	V & VI
Difference in mortality %	15.6	12.5	9.5	6.1	3.0	26.2	47.7	21.5
Value of X^2	13.85	10.82	8.61	2.33	0.74	57.90	202.60	25.09
Probability	*	*	*	0.14	0.41	*	*	*

*Probability against a difference as great as the one noted being due to chance is very great; much less than .01.

Another interesting point to be observed is that the differences between the various breeds are significant. Such differences, however, are probably not representative of the breeds as a whole, but more likely represent strain differences within breeds. More information on this point is desirable.

It is interesting to note in this connection, also, that the chicks from the Rhode Island Red x White Leghorn cross are slightly more resistant than the White Leghorn chicks and much more resistant than the Rhode Island Red chicks. This would seem to indicate a dominance of the factors for resistance carried by the White Leghorn and, likewise, to indicate that the Rhode Island Red may carry factors for resistance that the White Leghorn lacks. The evidence for the last point, however, is not conclusive, since a difference as great as that noted between the White Leghorn chicks with non-typhoid parentage and the crossbred chicks with similar parentage might occur over four times out of ten similar trials ($P = .41$) due to random sampling. These results indicate that multiple factors are operative in determining resistance to fowl typhoid and that different combinations of them exist in the various breeds and strains.

The Effect of Selection Upon the Rate of Mortality

Figure 1 shows the rate of mortality in the various groups of chicks. The percentage of survivors is plotted on successive days after inoculation beginning with the third day, the number of survivors on the third day being considered as 100 percent. Chicks dead before the third day were not considered in plotting the curves because only seven chicks out of the 1305 inoculated died before the third day, and most of these were listed as weak at the time of inoculation. Furthermore, an incubation period is necessary and since the heavy mortality began on the third day the writers felt justified in assuming that any mortality previous to this date was probably due to other causes.

It will be noted that the chicks from typhoid-surviving parents died at a much slower rate than those from non-tested parents. Here also, as in total mortality, the chicks that came from parents which had both survived typhoid infection, died at a slower rate than those that had only one parent that was a typhoid survivor. In these two groups but very little mortality occurred before the fifth day and the mortality was much more gradual than in any of the other groups. The mortality of chicks coming from non-tested parents commenced earlier, namely on the third day, and proceeded at a much faster rate. This indicates a higher potential resistance in the chicks having typhoid ancestry.

Some rather pronounced differences are to be noted in the mortality curves of different breeds as well as in total mortality. The Rhode Island Red chicks proved most susceptible as indicated by both rate of and total mortality. The White Plymouth Rock chicks proved most resistant of the three breeds, while the White Leghorns were intermediate. Crossbred chicks from White Leghorn mated with Rhode Island Red birds gave a slightly less total mortality than the White Leghorns, but their curve of mortality resembled more nearly that of the Rhode Island Red chicks.

The mortality curves of the chicks from the non-tested parents tend to flatten out toward the end of the twenty-first day. This is due mostly to the fact that but few chicks were surviving after the fifteenth day. A similar trend, but less pronounced, may be noted in the curve of mortality of the chicks with typhoid ancestry. In this case, however, many more chicks were living at the end of the fifteenth day, and hence a comparison of the two curves after this period, and even before, is not justifiable.

Mortality after the twenty-first day was not considered as being due to typhoid alone. Most of the chicks surviving to this date had recovered their appetite and activity, and with reasonable care a very large majority of them would have lived to maturity. At the end of three weeks it was necessary to transfer the chicks to poultry houses. The conditions in the latter were very unfavorable, due to crowding and the placing of chicks of different ages in the same house. But in spite of the poor conditions approximately 100 of the chicks grew to maturity.

DISCUSSION

The data presented herein have definitely shown that chicks descended from parents that have survived an acute infection of fowl typhoid have a much higher resistance to the same disease than chicks hatching from birds that have never been subjected to an attack of fowl typhoid. It may be suggested that other causes than inherent resistance are operative here. Two other possibilities present themselves in the group of chicks whose dams had survived the disease. The first of these is that a certain amount of passive immunity may be transmitted to the chick thru the yolk and albumen of the egg, and the other is that the ovary of the hen may be affected, in which case the chick may be infected before hatching and thus acquire a greater resistance.

In consideration of the first of the above possibilities it should be pointed out that in the group of chicks whose sire only was a typhoid survivor this possibility cannot be considered as a cause of greater resistance. The experiments of Smith (1907), Hadley (1914), Learmouth (1923) and others have conclusively shown that acquired immunity is not transmitted to the offspring by their sire. Since this group of chicks had a much higher degree of resistance than chicks from non-tested parents, and intermediate between the latter and the chicks with double typhoid-surviving parentage, some other factors than passive immunity must have been operative in causing this greater resistance.

Relative to the infection of the ovaries of hens that have survived an attack of fowl typhoid, it may be said that there is evidence that this is sometimes the case. Doyle (1926) found infected ovaries in four out of nine hens that reacted to the agglutination test for this disease, but in no case was he able to demonstrate the presence of the bacterium in the eggs from these hens. Likewise, he reports that chicks hatching from eggs from these same hens showed good viability. He further states that the clinical history of outbreaks of avian typhoid in Great Britain present strong evidence against its transmission through the egg. On the other hand, Beach and Davis (1927) have presented evidence which seems to indicate that the disease is transmitted through the egg. In no case did they find the bacterium in eggs laid by hens with infected ovaries, although the particular outbreak of the disease reported by them indicated that this was the manner of its transmission. Granted that transmission through the egg sometimes occurs, it would seem, from evidence presented, to be the exception rather than the rule.

It would appear, therefore, that transmission of the bacterium through the egg cannot have accounted for much of the increased resistance noted in this experiment. If such infection of the eggs were the rule rather than the exception, it is probable that the hatchability of the eggs of the typhoid-

surviving birds would have been markedly decreased. Such was not the case, for the hatchability from these birds was, on the whole, very good.

A summary of the separate matings in the group with double typhoid-surviving ancestry, as well as in the other matings, shows a great difference in the mortality of chicks from different males, and this is further reason for not considering these two possibilities as having played any major role in determining the greater resistance of their chicks to fowl typhoid. These data are shown in Table III. It will be noted from this table that the mortality for the males with double typhoid-surviving ancestry varied from 25.8 to 58.7 percent. In the males from non-tested parents it varied from 79.7 to 96.4 percent.

TABLE III. The influence of different sires on the mortality of their chicks when subjected to an acute infection of fowl typhoid.

Parentage of chicks	Sire's No.	No. of chicks	Number ¹ dead	Percent dead	Ave. date of death after infection	Breed
Typhoid Surviving						
♂ ♂ x ♀ ♀	2139	150	88	58.7	11.57	White Leghorn
"	2670	140	55	39.3	11.80	"
"	2570	120	31	25.8	11.65	"
Non-tested ♂ ♂ x ♀ ♀	18	84	80	95.2	7.35	"
"	19	55	53	96.4	7.47	"
"	22	57	50	87.7	8.63	"
* "	23	125	105	84.5	7.10	"
† "	24	84	71	84.5	7.68	"
* "	23	59	47	79.7	5.25	W. L. x R. I. R.
† "	24	45	42	93.3	5.53	"

¹Mortality based on the interval from the 3rd to 21st days, inclusive.

*These two groups had the same sire.

†These two groups had the same sire.

The probability for differences as great as the ones noted between the different typhoid-surviving sires being due to chance have been calculated by the X^2 method. The odds against differences as great as those noted between sires 2139 and 2670, and between 2139 and 2570, respectively, are extremely great, while between 2670 and 2570 the odds are also very great (over 43 to 1) against this difference being due to chance alone. According to Fisher, if the odds against any difference being due to chance equal 19 to 1 ($P = .05$) one is justified in assuming that some other cause than chance has produced the difference observed.

Each of these males was mated with a sufficiently large number (approximately 12 each) of females to represent a fair sample of the population. Hence there is little likelihood that infection of the egg previous to the time of laying can have played any great part in causing the marked differences in resistance of the chicks from these males to the fowl typhoid bacterium. Rather, it is probable that these differences are due to variations in the genotypes of the males concerned.

One other point of interest appears in Table III. It will be observed that the average date of survival after inoculation of the chicks from males that were typhoid survivors is much longer than that of the chicks from

males who were not typhoid survivors. It is to be noted, also, that the average date of mortality after inoculation is considerably earlier in the cross-bred chicks (White Leghorn x Rhode Island Red). These chicks died on the average in a shorter time after infection than did the White Leghorn chicks from non-typhoid-surviving parents, although their total mortality was less. (See Table I.) This same relationship is shown in another manner in Figure 1.

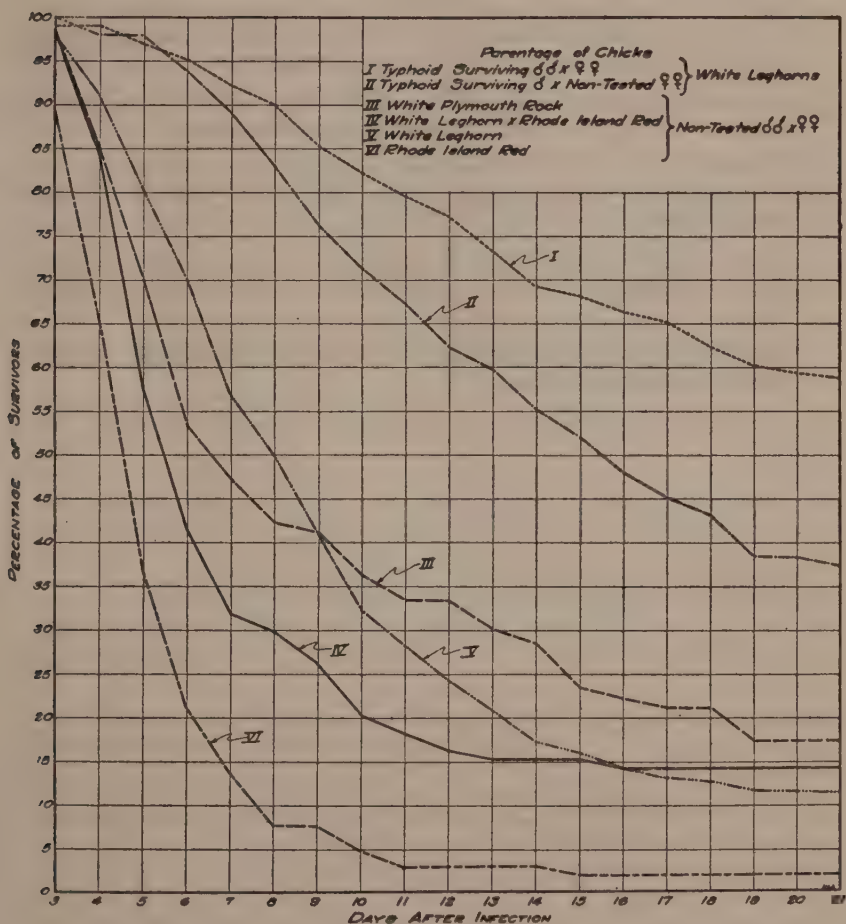


FIG. 1. THE PERCENTAGE OF CHICKS OF THE TOTAL POPULATION SURVIVING ON GIVEN DATES AFTER INFECTION

SUMMARY

1. Chicks hatched from parents that had both survived an acute infection of fowl typhoid gave a total mortality of 40.9 percent.
2. Chicks whose sire only had survived such an infection showed a mortality of 62.4 percent.
3. Chicks from non-tested parents showed a mortality ranging in different breeds from 82.5 to 98.1 percent.
4. The mortality rate in the groups from non-typhoid-tested parents was also much greater than that in the other two groups.
5. The mortality rate in the chicks with single typhoid ancestry was greater than that of chicks with double typhoid ancestry.
6. Evidence is presented to show that the lower rate of mortality in the chicks hatching from typhoid-surviving parents cannot be due to chance alone.
7. Significant differences were noted in the mortality of different breeds, but it is suggested that these represent strain rather than breed differences.
8. Multiple factors are undoubtedly concerned in determining resistance to fowl typhoid in chickens.

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STUDIES IN HOME CANNING*

I. *Some Factors Affecting the Keeping Qualities of Vegetables and Meats Canned by the Hot Water Bath Method***

By GERTRUDE SUNDERLIN WITH P. MABEL NELSON AND MAX LEVINE

From the Departments of Foods and Nutrition and Bacteriology.

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INTRODUCTION

Although the hot water bath method of canning vegetables and meats has been employed in the home for many years, a survey of recommended procedures discloses an extreme lack of agreement as regards the period of heating necessary to produce products which will keep satisfactorily. Thus a perusal of bulletins published by the Extension Departments of 21 different states since 1924 indicates the following variations in the heating periods recommended:

Asparagus	1	-3	hours
Beans	1¼	-3	hours
Beets	1	-2	hours
Corn	1½	-4¼	hours
Greens	1	-3	hours
Peas	1½	-3½	hours
Squash	1	-5	hours
Tomatoes		12-45	minutes

The experience with botulism from canned foods has naturally raised a question as to the efficacy of the method formerly recommended for canning both home and commercially prepared products and has led some to look with disfavor on the hot water bath method. Thus the United States Department of Agriculture, Bureau of Home Economics (1924) and Stanley (1926a) recommended that the pressure cooker (autoclave) be used for all vegetables except tomatoes, so as to reduce "spoilage difficulties and the risk of poisoning from occasional contamination with *botulinus* bacteria." Again Stanley (1926b) stated that "special work has been done on canning asparagus and different varieties of beans and the spoilage records substantiate our earlier conclusion that these vegetables should be processed under pressure," but unfortunately no data were presented to substantiate this conclusion.

*This forms part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Iowa State College.

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It may be questioned if the steam pressure cooker would be more efficient in the hands of an untrained housewife than the boiling water method with an adequate processing period. The variation in the heating periods recommended for home canning of vegetables has already been referred to. This, together with the scarcity of published data on the efficiency of the water bath method of food preservation, led to the studies reported in this paper.

HISTORICAL

As early as 1909, the United States Department of Agriculture published a bulletin which gave directions and time tables for home canning of non-acid vegetables by the hot water method. (Breazeale, 1909). In spite of the fact that the home canning of vegetables and meats increased tremendously at the time of the war and that time tables for processing by the hot water bath method have been recommended by the U. S. Bureau of Home Economics and the extension workers in the different states, there are very few experimental results published to show the basis for the recommendations made. The available published data on the efficiency of the hot water bath method of canning are reviewed below. No attempt has been made to include the immense canning literature of the commercial field or writing on home canning where experimental data are not given.

Normington (1919) reported results from the canning of 213 jars of peas by the cold pack method, one lot processed in streaming steam, five in the hot water bath and seven at 15 pounds pressure. The proportion of spoilage varied from about 6 to 100% on the different days. Of those autoclaved at 15 lbs., 50.9% spoiled, whereas those cooked 3 hours in the hot water bath showed 63.9% spoilage. On all but one day the peas were left overnight, after gathering, before they were canned. The one time the canning was done on the same day the peas were picked gave a lower percentage of spoilage (12.1% for 40 min. at 15 lbs.). In view of the fact that the freshness of material canned has an important bearing on the keeping qualities, it would be necessary, in comparing the two methods of processing, to restrict the comparison to material of the same degree of freshness. If, therefore, the one batch which was canned fresh is eliminated from the comparison, the proportion spoiled is found to be 63.9% in the hot water bath and 66.7% for that canned at 15 lbs. pressure. These figures are not strictly comparable as some of the processing was done in pint jars and some in quarts and the proportionate number of each was not always given. From the evidence she presented it appears that there was no choice between the pressure cooker and the hot water bath from the standpoint of spoilage.

Skinner and Glasgow (1919)* made an extensive study of the canning of asparagus (664 pints), observing such variables as time of process, different methods of intermittent processing, addition of salt and addition of acid. They found that the 3 hour process without acid showed 50% spoilage, the 2 hour boiling a still greater loss and all processed for less than 2 hours spoiled. They suggested adding one tablespoon of 4.4% vinegar to each quart of water used to fill the jars. With this addition, a 2 hour process was found sufficient for the asparagus to keep.

Margaret MacFarlane (1919) reported results of experiments in can-

*Also reported in Kansas Experiment Station Report, 1919, p. 76.

ning 459 pints of vegetables, using the one period hot water bath process, the intermittent three day process and pressure cooker process at 10 and 15 lbs. With 11 different vegetables and several process methods there were necessarily only a small number of jars of each vegetable canned by each method. In many cases only two or three jars were employed for each variable, the largest number being 40. She recommended intermittent sterilization for asparagus, beans, corn and peas; the one period process 1 hour for beets and cauliflower, 2 hours for beet greens, carrots and Swiss chard, and 22 minutes for tomatoes. The recommendations were based on no spoilage in the following number of jars: asparagus 15, beans 22, beet greens 3, cauliflower 5, corn 15, peas 16, Swiss chard 6, tomatoes 30. In case of beets, 8 of the 26 jars canned by the recommended method spoiled and in the case of carrots there were 2 spoiled out of 25 prepared, but this spoilage was attributed to factors other than length of time of processing.

Biester, Weigley and Knapp (1921) canned 175 jars of vegetables, including beet greens, beets, beans, carrots, corn, tomatoes and pumpkin, by the cold pack method. They noted the effect of storage temperature upon keeping quality. Of 113 jars kept in a cellar at a temperature from 0 to 23° C. only 9 spoiled, while 33 of the 62 jars kept in the incubator at 28° to 32° C. spoiled.

Edmondson, Thom and Giltner (1922), in work with boric acid canning powder, canned one series of vegetables according to the "one period cold-pack" method of Farmers' Bulletin 839. Of the vegetables canned in this way they had spoilage from one of four jars of corn, none of eight asparagus and one of four string beans, none of two lima beans and two of two peas. The others they canned were inoculated with organisms or canned with the addition of canning compound and therefore are not pertinent to this investigation.

Levine (1923), in the course of a series of experiments on the value of boric acid canning compound in food preservation, gave spoilage records for a number of jars canned according to the "cold pack" method of Farmers' Bulletin No. 839, U. S. D. A. Of 31 jars of asparagus, carrots, corn, string beans and green peas processed the recommended time lengths only one jar of corn was spoiled. The temperature of incubation was from 19° to 37° C. The material was exceptionally fresh, being gathered immediately before canning, the longest time elapsing between picking and the beginning of processing being 3 hours in the case of corn. This might explain the extremely small amount of spoilage.

In 1924 an extensive investigation was carried on with commercial processing of tomatoes. Part of the results would be applicable to home conditions. Esty (1925) reported briefly the results of this work. There were 15,000 cans of tomatoes inoculated with spoilage organisms and processed for different times under different conditions. The temperature and time necessary to sterilize was largely dependent upon the initial contamination. Pressure cooking was thought to offer no advantage over the hot water bath.

Abbott (1926) reported work done on the canning of peas and corn. Two processes were used: 3 hours in the hot water bath and 50 minutes at 10 lbs. pressure. The peas processed by either method spoiled and the corn did not. He stated that the packing method may have been a factor, as the corn was packed very loosely and the jars were filled with hot water, which made the heat penetrate more quickly to the center of the jars.

Considerable work on the value of acid in the canning of vegetables has been done in the laboratory of the University of California by Cruess and his co-workers. Experimental work of Cruess (1916) showed that peas, beans, pumpkins, beets, turnips, artichokes and asparagus processed 1 hour in a brine to which lemon juice had been added kept perfectly, while those without the acid spoiled. He recommended from 4 to 8 oz. lemon juice per gallon of brine for the various products and a process of from 45 minutes to 1 hour in cans and 1 hour to 1½ hours in jars. The details of the experiments were not reported. In a later publication (1925) he recommended 16 tablespoons (8 oz.) strong vinegar or lemon juice per gallon of water used to can asparagus, green beans, beets, carrots, turnips, parsnips and onions and 8 tablespoons for meats, with a process time varying from 2½ hours for beets to 4 hours for most of the vegetables and meats. Although he recommended the same concentration of acid as in 1916, he stipulated a much longer process time than formerly recommended.

Cruess, Fong and Liu (1925) carried on extensive experiments to show the role of acidity in vegetable canning. Their work was done with vegetables inoculated with *Cl. sporogenes*, *Cl. botulinum*, and a heat resistant thermophile, with brines made up to different pH values by addition of acetic, hydrochloric and citric acids. They found the effect of pH value on heat resistance of these spores to be very pronounced and that it was possible to sterilize canned vegetables much more easily in acidified brines than in non-acidified brines.

Fong (1926) concluded that the final reaction of corn, beans, spinach and asparagus which had been acidified must be pH 5.0 or less to greatly decrease the death time of *Cl. sporogenes*, *Cl. botulinum* and the thermophile which he employed. When the vegetables were processed for not less than one hour at 212° F., the reaction of the original brine which was found to be dependable for the prevention of spoilage by *Cl. botulinum*, *Cl. sporogenes*, and the thermophile was pH 2.8 to 3.0. In jars inoculated with *Cl. sporogenes* and processed one hour at 212°, he had no spoilage of sweet corn when the original reaction of the citric acid brine was pH 3.2 and the final pH 5.0, no spoilage of string beans when the original reaction was pH 3.6 and the final pH 5.0, no spoilage in spinach when the original reaction was pH 2.2 and the final pH 4.0, and no spoilage in asparagus when the original reaction was pH 3.6 and the final pH 4.6. With more alkaline reaction or a shorter process time, spoilage resulted.

EXPERIMENTAL

The work reported here is restricted to observations on the hot water bath method, with a view to securing spoilage data and time tables for this method of canning vegetables and meats. The vegetables were canned during the summer of 1926 and 1927 and the meat in the winter of 1927.

The canned products were stored for a period of from 5 to 8 months at temperatures varying from 21 to 29° C. (average about 24° C.). Conditions of canning were controlled as far as was possible. The variety and condition of the product, weather conditions, pH of the water used, barometric pressure and bacterial count of the product as it went into the boiler were noted.

One jar from each boiler was opened as soon as possible after processing to determine the original condition of the product, to serve as a basis

for comparison later. The general appearance, odor, suction, pH, titrable acidity, ammonia and amino nitrogen as determined by the Sörensen formol titration, microscopic examination of the sediment and bacterial counts on plates at 37° C. and 20° C. and in dextrose broth tubes were noted. The jars on the shelves were observed at regular intervals and tests as described above were made on the products as they spoiled. The jars which had not shown evidence of spoilage at the end of the storage period were opened and tested as above.

Jars which gave evidence of bacterial growth or change due to bacterial growth by organoleptic, chemical or bacteriological tests were considered to be spoiled. Jars which did not have a perfect seal at the time of canning, as evidenced by the failure of the new zinc caps to be drawn down by suction, were not included in evaluating the relation of period of processing to spoilage. Such imperfection was found likely to be a cause of spoilage irrespective of the process time.

TABLE I. The record of 3040 jars of vegetables and meats canned during 1926-1927.

	Jars canned	Broken or discarded	Examined† as controls	Jars stored
Pork	288	6	20	262
Beef	336	11	24	301
Tomatoes	144	6	12	126
Asparagus	230	5	18	207
Beans 1926	484	11	39	434
Beans 1927	532*	5	31	472
Chard 1926	120	1	10	109
Chard 1927	264	0	21	243
Sweet Corn 1926	294	3	30	261
Sweet Corn 1927	348	2	29	317
Total	3040	50	234	2732

*24 jars in storage at time of preparing this paper.

†These were opened soon after preparation and examined bacteriologically and chemically to serve as a basis for comparison.

GENERAL PROCEDURE

The variations in the methods followed in the canning of different foods are detailed below. In general, the canning was carried out in the following manner: By previous arrangement with the gardener, the vegetables were gathered and brought immediately to the laboratory. They were thoroughly washed through several waters and cut for packing. The desired amount of vegetable for each jar was weighed and then precooked by adding a measured quantity of boiling water and cooking in a small uncovered sauce pan over a gas burner for five minutes. (*Precooking* is the term used to designate the process whereby the product is thoroughly heated before being put into the jars and the water in which it had been heated is used to fill the jars.) The precooked product was packed into the hot jar as quickly as possible. If the liquid on the product did not fill the jar to within $\frac{1}{4}$ inch of its top, boiling water was added. A hot cap was screwed on until the seal was within $\frac{1}{2}$ inch of completion. The jars were submerged in sufficient boiling water to cover the caps to a depth of at least three inches and timing was started when the water reached the

boiling temperature. The processing consisted of keeping the jars covered by boiling water for definite lengths of time. After the desired periods of processing, the jars were removed, the seal completed at once, and the jars inverted. They were left in the laboratory overnight and the next morning examined for imperfect seals, labeled and stored. Pint Ball Mason jars were used. New Ball rubbers and new Ball zinc caps were used except where otherwise indicated. Twelve jars were processed at one time in a boiler. Individual racks held the jars.

On the days when the materials were canned, the theoretical boiling point of water varied from 98.87°C . to 99.22°C . due to differences in the barometric pressure.

ASPARAGUS

Methods used.—In the control method with asparagus, the product was scalded and the water discarded, fresh boiling water being used to fill the jars. The scales were removed from the stalks, except at the tip, during the cleaning of the asparagus. Three hundred gram portions of asparagus were used to each pint jar, making a fairly loose pack. Preliminary experiments were made to ascertain the difference in flavor of the asparagus packed cold, precooked and scalded. The asparagus that was scalded and the water discarded was mildest in flavor. Since canned asparagus is considered by some to be too strong in flavor, scalding rather than precooking was chosen for the control method. (Scalding differs from precooking only in that the water used is drained off and discarded and fresh boiling water added to fill the jars.) It was also thought that discarding the scalding water might discard enough bacterial spores to make some difference in the keeping qualities of the product. On one day, half of the jars were filled with scalded asparagus and half with the precooked product. Aluminum caps were used on half the jars in each boiler throughout the canning of asparagus.

The asparagus canned with the addition of acid was treated in the same manner except that the boiling water added to the jar contained 2.0 c.c. of 5 normal phosphoric acid or 1.5 c.c. of 5 normal citric acid for 220 c.c. of liquid used. Preliminary trials indicated that these respective quantities of acid could be added without detrimental effect on the flavor of the product. The 220 c.c. of liquid was added to each jar of scalded asparagus. The reaction of the liquid before processing was pH 3.4 with citric acid and pH 2.2 with phosphoric acid. After processing, the liquid in the case of the non-acidified asparagus showed a pH of 6.0 to 6.2, while that with either citric or phosphoric acid added was pH 5.1. After seven months storage the liquid in the "no acid" jars was pH 5.8-6.0 and that in the acid jars was pH 5.6.

Results.—Asparagus could not always be secured in large enough quantities to can all the jars of one series on the same day. On each day at least ten jars were canned by the control method. There was more variation in the spoilage obtained on different days with these control jars than for the different process times on any single day. For instance, there was no spoilage in the 2 hour process of those canned on June 3d, while on May 11th there was 10% spoilage and May 18th, 78.6%. For this reason the summary of spoilage with different lengths of process must be employed with caution. Apparently some undetermined factors influ-

enced the keeping qualities of the asparagus. The results are summarized in Tables II and IIA.

TABLE II. Showing spoilage records of 207 jars of asparagus canned under different conditions.

Process time in hours			3	2½	2	1½	1
Variable observed	Date canned	No. jars per process time	Percentage spoiled				
Effect of removing scales from stalks							
Scales removed	5/18	14	35.7		78.6		
Scales not removed	5/18	14	35.7		64.3		
Effect of precooking rather than scalding							
Scalded	6/3	10			0		
Precooked	6/3	11			0		
Effect of addition of acid							
No acid (control)	5/14	10		10			
Phosphoric acid	5/14	10, 10, 11			0	0	36.4
No acid (control)	5/23	10	30				
Citric acid	5/23	11, 9, 11			0	11.1	9.1
Effect of batch of material							
Batch canned on	5/11	10			10	50	
* " " "	5/14	10		10			
" " "	5/16	14		28.5		92.9	
* " " "	5/18	14	35.7		78.6		
* " " "	5/23	10	30				
* " " "	6/3	11			0		

*Noted also in sections above.

TABLE IIA. Summary of asparagus canned without acid.

Process time in hours	3	2½	2	1½
Number jars stored	38	24	59	24
Number jars spoiled	13	5	21	18
% spoiled	34.2	20.8	35.6	75.0

Although the removal of the scales from the stalks eliminated considerable dirt, and consequently large numbers of bacteria, such cleansing did not make a significant difference in the percentage of spoilage on the day this factor was used as a variable. From a bacterial count of the dirt washed off in removing scales from two stalks, it was estimated that about 4,000,000 more bacteria were added to the jars when the scales were not removed. However, the scales from the tips were not removed in any case and it is possible that enough dirt was left in them to make the amount removed of no significance.

Precooking, as compared with scalding, made no significant difference in the keeping qualities of asparagus under the conditions that prevailed on the day of the experiment. Bacterial counts of discarded scalding water showed about 500 bacterial spores per c.c. of water. It may be that the plant acids discarded at the same time counterbalanced the advantage of having a product with fewer spores to kill. The pH difference of the two products was not discernable colorimetrically, but the titrable acidity was slightly higher in the precooked jars.

The addition of acid had a decided effect on the keeping of asparagus. Both the phosphoric and the citric acid series were run on days when spoilage in the "non-acid" controls showed up in longer process times than were employed for the acid series. There was no spoilage in the 2 hour process when citric acid was added, while the 3 hour "no-acid" series showed 30% spoilage on the same day. There was no spoilage in the 1½ or 2 hour process when phosphoric acid was added, while the 2½ hour "no-acid series of that day gave 10% spoilage.

Since the keeping of asparagus in the 3 hour hot water bath process is partially dependent on some undetermined factor, it would seem advisable to use the 2 hour process with the addition of acid when the hot water bath method is employed.

BEANS

The control method used for canning beans was the same as outlined in the general method for vegetables except that in 1927 the precooking time was shortened to three minutes. For each pint, 340 grams of green

TABLE III. Showing spoilage records of 472 pints of green beans canned under different conditions (1927).

Process time in hours	2½	2	1½	1	
Variable observed	Percentage spoiled*				
Effect of precooking, scalding or packing cold					
Packed cold	0 (10)	0			
Precooked	0	0			
Scalded	0	11.1 (9)			
Effect of delay in processing after precooking					
Processed immediately	9.1		36.4		
Processed after 2 hours	0		0 (12)		
Processed after 4 hours	0 (12)		8.3 (12)		
Effect of storage temperature					
Basement 12-21° C.	0	0			
Room 21-28.5° C.	0	0			
Incubator 37° C.	66.6 (12)	100 (12)			
Incubator 55° C.	100 (12)	100 (10)			
Effect of character of caps and rubbers					
Half aluminum caps, half zinc	0 (23)	0 (23)			
Caps from 1926 spoiled beans	16.6 (12)	25.0 (12)			
Rubbers used once in 1926	0 (10)				
Effect of source of beans					
Secured from B	0 (7)	42.9 (7)			
Secured from H	0 (7)	14.3 (7)			
Effect of consistency of pack					
Loose pack	0	0			
Solid pack	0	0 (10)			
Effect of addition of acid					
No acid added		0	9.1	45.5	
Citric acid added		0	0	0	
Phosphoric acid added		0	0	9.1	

*Percentage based on 11 jars except where otherwise indicated by numbers in ().

beans were used in 1926, 300 grams of yellow beans in 1926 and 275 grams of green beans in 1927. The beans canned in 1926 were stored for a period of about eight months and those canned in 1927 for five to six months. The variables, aside from time of processing, were: various methods of "pre-treatment" (scalding, precooking or packing cold), delay in processing after precooking, storage temperatures, caps and rubbers, source of product, tightness of the pack, addition of acid, delay in completing the seal, and freshness of the product. The results are summarized in Tables III, IV and IVA.

TABLE IV. Showing spoilage records of 434 pints of beans canned in 1926 under different conditions.

Process time in hours	4	3½	3	2½	2	1½
Variable observed	Percentage spoiled*					
Effect of storage before canning						
Canned fresh	0	0	4.5	0 (10)	22.7	70 (10)
Canned after 1 day	9.1	33.3 (21)	13.6	30 (10)	18.2	54.5 (11)
Canned after 3 days	0 (18)	9.1	13.6	36.4 (11)	42.9 (21)	27.3 (11)
Canned after 5 days	18.2	28.6 (21)	59.1	100(5)	73.3 (15)	
Effect of time elapsing before completion of seal						
Seal completed after 15 sec.	0 (4)					
Seal completed after 30 sec.	0 (4)					
Seal completed after 45 sec.	0 (4)					
Seal completed after 60 sec.	0 (16)					

*Percentage based on 22 jars where not otherwise indicated by number in ().

TABLE IVA. Summary of beans canned under ordinary conditions.*

(From Tables III and IV)							
Process time in hours	4	3½	3	2½	2	1½	1
Number jars stored	50	22	22	144	144	32	11
Number jars spoiled	0	0	1	1	10	12	5
% spoiled	0	0	4.5	0.6	6.9	37.5	45.5

*Excludes those stored at incubator temperature, those treated with acid, those canned with caps from spoiled beans and those stored before or after precooking before processing.

Effect of scalding, precooking or packing cold.—To ascertain the effect of methods of treatment before processing the following series were prepared:

(1) 24 jars were filled with beans which had been precooked for 3 minutes. These served as controls.

(2) 24 jars were prepared with beans that had been scalded for the same length of time. The water had been discarded and the jars were filled with boiling water.

(3) in 24 jars the beans were packed cold and covered with boiling water.

Scalding, precooking or packing cold made no significant difference in the keeping of the beans.

Effect of delay in processing after precooking.—To ascertain the effect of delay in processing, 24 jars were processed immediately after precooking, 24 were allowed to stand in a warm room (at about 26° C.) for two hours and another 24 for four hours. A temperature record was taken of the cooling curve of the beans after precooking. At the end of one hour the temperature was 52° C., at the end of two hours 39° C., and at the end of three hours 31° C. The heating curves of the cooled beans and the beans processed while hot were not taken as Magoon and Culpepper (1922) have shown that water bath temperature was reached only six or seven minutes later when quart jars of string beans were started at 20° C. than when they were started at 80° C. The difference would be still less in pint jars.

The bacterial count taken from one jar of the beans changed, on standing, from 4 per c.c. in the precooked material to 25 per c.c. in the same after two hours and to 40 per c.c. after four hours. The two and four hour liquids, after being brought to boiling, gave a count of 8 and 3 per c.c., respectively.

The delay in processing after precooking had a favorable effect on the keeping of the beans. The beans that were held for two hours after precooking before processing showed no spoilage in the 2½ or 1½ hour process, while those that were processed immediately showed 9.1% and 36.4% spoilage, respectively. This might appear at first thought contrary to our usual conceptions. These results might be explained in the following manner. The vegetative cells were killed, for the most part, by the precooking. Many of the spores might germinate during the time elapsing before beginning the final processing, thus reducing the number of resistant cells to be destroyed. Similar experiments would have to be carried out with beans on different days and beans from many sources before one could say that holding for a definite interval after precooking would be likely to reduce spoilage. The number of jars canned was not large enough to warrant definite conclusions, but the results indicate that experiments along this line might be illuminating.

Effect of storage temperature.—The beans that were to be stored at different temperatures were canned according to the control method. Three jars from each boiler were taken to make up the 24 stored at each temperature range. Those stored in a basement were in a private home in the vegetable room opening from the furnace room. The temperature in August was as high as 21° C. in this basement room and the lowest temperature recorded was 12° C. Those stored at room temperature were subjected to a range of from 21 to 28.5° C., the average being about 24° C. This room, a small inside storage room in the basement of Home Economics building, was used to store the other canned products. The third series was stored at 37° C. and the fourth at 55° C.

The temperature at which the canned beans were stored had a decided effect on the keeping qualities. No difference was observed between the basement and room temperatures, since there was no spoilage at either range in the 2½ or the 2 hour process. However, at 37° C. and 55° C. the amount of spoilage was 66 and 100%, respectively. This indicates that sterility was not obtained, although the process time was sufficient to yield keeping products when they were stored at lower temperatures.

Effect of character of caps and rubbers.—In this series 24 pints of beans were canned with new aluminum caps, 24 pints with new zinc caps,

24 pints with caps from jars of beans which had spoiled in 1926 and 12 pints with rubbers that had been used in 1926, but were not from jars that had spoiled. The caps from the spoiled beans had been washed when the beans were opened and had been kept at room temperature. They were washed again and boiled five minutes before using.

The beans that were canned using aluminum caps instead of zinc kept equally well. Re-use of rubbers that had been used in 1926 made no difference in securing a perfect seal or in the keeping of the jars thus treated. Using caps that had been taken from jars of spoiled beans in 1926 made a distinct difference in the keeping of the product. Thus, whereas jars processed with new caps for $2\frac{1}{2}$ and 2 hours showed no spoilage, those in which the old caps were employed gave 16.6% and 25% spoilage for the respective process periods.

Effect of source of beans.—The beans in 1927 were secured from two gardens ("B" and "H"). On one day the beans were secured from both gardens to get a comparison of the spoilage. The comparison involved another factor, however, as the beans from "B" were more mature than the beans from "H". This difference in maturity was observed with the other beans from these sources.

The spoilage results were slightly different with the beans from the two gardens, although not as different as the results on the day when this was employed as the variable would indicate. Considering all of the 2 hour process beans (1927) canned by comparable methods, 61 pints were from beans secured from garden "B" and 61 from garden "H". Of these there was 6.3% and 1.6% spoilage, respectively. On the day "source" was used as a variable there was a higher percentage of spoilage than usual in the beans secured from both gardens.

Effect of consistency of pack.—In the series with variation of pack, 212 grams of beans per pint were used in the loose pack and 425 grams in the solid pack. The loose pack required more water to fill the jars after packing, while the solid pack was so tight that there was little room for water.

The consistency of the pack made no difference in the keeping qualities of the beans canned. Heat penetration figures obtained by Redfield (1927) showed a difference of at least 30 minutes in the time required for the loose and solid pack to reach the process temperature. From these figures it would be expected that at some process time lengths there would be a difference in keeping qualities, but a short enough time was not used to show this in these experiments.

Effect of addition of acid.—The beans that were canned with the addition of acid were treated and processed according to the control method except that 1.2 c.c. of 5 normal phosphoric acid or 0.8 c.c. of 5 normal citric acid were added to the cup of water used in precooking the contents of each jar. The reaction of the acidified water was pH 4.0 with the citric and pH 2.6 with the phosphoric acid. After processing, the reaction of the liquid on the non-acidified beans was pH 6.0, while that with citric or phosphoric acid was pH 5.3. After five months storage the reaction of the liquid in the non-acidified beans was pH 5.4, while that in the acidified was pH 5.3-5.4.

The addition of acid decreased the time necessary for processing the beans. There was no spoilage in the $1\frac{1}{2}$ hour process when acid was

added, but on this day there was only 9.1% spoilage in the 1½ hour "no-acid" beans. In the 1 hour process, there was 45.5% spoilage in the "no-acid" beans, none in those to which citric acid had been added, and 9.1% in those to which phosphoric acid had been added. One and one-half hours was a satisfactory process time for the beans canned with the addition of acid.

Effect of storage before canning.—During 1926, two series of experiments were run to determine the influence of the freshness of the product on the keeping qualities. Enough beans for 240 pints were secured at one time. Sixty pints were canned soon after they were gathered. The beans that were kept for one, three and five days before canning were stored in market baskets in a basement room with a temperature range of from 22 to 26° C. The beans that had stood one day required ⅛ cup more water to fill the jars than the fresh beans. They appeared to be in good condition. The three day old beans were in very poor condition, some molded, some with decomposing areas, some dried and shrivelled, and the color was changed from a deep green to a yellowish green. They were tough and hard to snap. They required ¼ cup more water to fill the jars than the fresh beans. The five day old beans were in much the same condition as the three day, but showed more decomposition and drying.

The beans which were canned when fresh and processed 2½ hours or longer showed little spoilage. The beans that had stood one day or longer before they were canned showed considerable spoilage although the percentage did not increase in any regular order. The relation of the freshness of the product, process time and keeping qualities can be seen from this table.

TABLE V. Showing relation of freshness of beans to keeping qualities.

	Canned fresh	Canned after 1, 3, 5 days
Processed 2½ hours or more	1.3%*	37.5%
Processed 2 hours or less	23.4%	41.3%

*Percentages represent spoilage.

Effect of time elapsing before completion of seal.—Twenty-eight pints of beans were canned with a view to noting the effect of a slight delay in completing the seal after the beans came from the boiler. Occasionally the last jars removed from the boiler are uncovered for a short time by the water level going below the rubbers as other jars are lifted out. This experiment was carried out to see whether those that have the seal uncompleted for a time have as good suction and as good keeping qualities. With 4 jars the seal was completed in 15 seconds after removing from the boiling water, with 4 in 30 seconds, with 4 in 45 seconds and with 16 in 60 seconds.

The lapse of one minute after the jar is taken from the boiler before the completion of the seal was not enough to make any difference in the suction or keeping of the product.

Summary of beans canned under ordinary conditions.—Of the 238 pints of beans canned under ordinary conditions and processed for 2½ hours or more, there were only two pints which spoiled (0.8%). Of 144 pints which were processed for 2½ hours only 0.6% spoiled. With lower periods of heating the amount of spoilage was very much higher: the 2, 1½ and 1 hour process periods showed spoilage to the extent of 6.9%, 34.4% and

45.5%, respectively. For the beans canned under ordinary conditions, 2½ hours was a satisfactory time for processing.

The spoilage data from 906 pints of string beans canned under different conditions indicate that string beans can be processed in the hot water bath without having a high percentage of spoilage if the storage temperatures are not high, if the beans are canned soon after gathering, and if reasonable precautions are taken against adding large numbers of bacteria to the jars.

SWEET CORN

The results of the observations on sweet corn canning are summarized in Tables V and VA.

The corn was cut in the Maine style, the tips of the kernels being cut from the cob and the milk scraped out. The corn for each jar (320 grams) was mixed with 160 grams of boiling water and brought slowly to boiling. It was packed into the jar as hot as possible and processed at once. The jars were stored and observed for a period of about eight months for the 1926 series and five months for the 1927.

Effect of storage before canning.—In 1926, two series were run where freshness of the product was a variable. The corn for three days canning was brought in at one time. One portion was canned immediately, one portion was canned after one day and one after two days. It was kept in bushel baskets in a basement room at about 23 to 25° C. The corn after one and two days storage had heated, and when canned the temperature inside the ears was from 28 to 35° C. It smelled slightly sour as it was cut from the cob. After the corn was canned the reaction of that canned after storage was 0.2 to 0.4 of 1 pH unit lower than that of the fresh corn. The bacterial count of the corn increased greatly on standing, as shown below:

		Bacterial Count	
		(Before precooking)	(After precooking)
A Series	Fresh corn	54,000 per c.c.	50 per c.c.
	1 day	825,000 " "	2 " "
	2 day	1,375,000 " "	240 " "
B Series	Fresh corn	31,000 " "	10 " "
	1 day	230,000 " "	6 " "
	2 day	190,000 " "	2100 " "

In 1927 one series consisted of corn that had been gathered the afternoon before and stored over night in the basement room at 23° C. as compared with a control series which consisted of corn from the same garden gathered by 9 o'clock the next morning and the processing begun within three and a half hours. The temperature of the freshly gathered ears of corn was about 18° C., while that of those stored overnight was between 28 and 30° C. There was no change in the pH of the canning mixture discernable by the colorimetric method. The bacterial count was increased by the storage, as indicated below.

		Bacterial count	
		(Before precooking)	(After precooking)
Fresh corn	3,000 per c.c.		5
Stored corn	640,000 per c.c.		20

In the three series designed to ascertain the effect of storage before canning the results obtained varied. In series "A" 1926, there was three or four times as much spoilage with the fresh corn as with that which had stood one day before canning. In series "B" the opposite was true. The fresh corn kept much better than the corn that had been stored one day. In 1927, the series "C" that was run with corn stored over night before canning showed more spoilage with the corn gathered in the morning than with that gathered the night before. Some of these results are contrary to what would ordinarily be expected. There are many factors involved. The pH change was similar in the "A" and "B" series, so would not have been the controlling factor. It seems that a possible explanation may be that in some cases the bacteria present are in more resistant form than in others. In series "A" it is possible that the bacteria, even though greatly increased in number after one day, were of a less resistant form at the time of processing. Thus the fresh material showed a bacterial count of 54,000 per c.c., which dropped on pre-cooking to 50 per c.c., whereas the material which had been stored for one day had an initial count of 825,000, which was reduced to 2 per c.c. through the simple process of precooking. This would indicate that although there were more organisms present in the "one day" material there were actually fewer heat resistant or spore forms. After two days storage the bacterial count rose to 1,375,000, which dropped to 240 per c.c. as a result of precooking. On the basis of the relative incidence of resistant forms as indicated by the count after precooking the amount of spoilage would be expected to be greatest in the "two day" material and least in the "one day". This was in line with the results obtained. This line of reasoning is not adequate to explain all of the results, however, for in the "B" and "C" series the amount of spoilage was not correlated with the number of organisms remaining after precooking. We must assume, therefore, that the resistance of the organisms surviving the precooking process, as well as the numbers, is a determining factor.

On the basis of the results obtained, one could not predict in advance whether storage would have a favorable or unfavorable effect on the keeping qualities of corn. There would be no way of determining, previous to canning, whether or not the bacteria were in the most resistant stage, so until further work is done along this line the assumption that fresh material keeps better is the better one to follow.

Effect of delay in processing after precooking.—In this series the corn was precooked and packed into jars. Twenty-four jars were processed immediately, 24 were allowed to stand 3 hours, 24 were allowed to stand 6 hours and 24 were allowed to stand 15 hours before processing. Cooling and reheating temperature curves were noted. In one hour the precooked corn had cooled to 54° C., in two hours it had cooled to 44° C. and in three hours to 32° C. The room temperature was 29° C. It took 95 minutes for this cooled corn to reach the processing temperature after it was placed in boiling water while the corn packed hot and processed at once reached the processing temperature in 40 minutes. The bacterial count before pre-cooking showed 7800 per c.c. of liquid, while afterward it showed 4 per c.c. The count in the same jar changed to 10 at the end of 3 hours standing, 165 at the end of 6 hours, and 22,800 at the end of 15 hours. The liquid from the 15 hour sample when boiled showed only 3 per c.c.

TABLE V. Showing spoilage records of 578 pints sweet corn canned under different conditions.

Process time in hours	4	3½	3	2	1½
Variable observed	Percentage spoiled				
Effect of storage before canning					
Series "A" (1926)					
Canned fresh	36.4	36.4	54.5	100*	
Canned after 1 day	9.1	9.1	9.1	63.6	
Canned after 2 days	81.8	72.7	80*	100	
Series "B" (1926)					
Canned fresh	9.1	0	0	72.7	
Canned after 1 day	40.0*	27.3	27.3	72.7	
Canned after 2 days	9.1	36.4	45.5	45.5	
Series "C" (1927)					
Canned fresh	18.2		9.1	63.6	
Canned after overnight storage	0		18.2	27.3	
Effect of addition of acid (1927)					
No acid added			100	100	81.8
Phosphoric acid added			0	0	18.2
Citric acid added			0	0	9.1
Effect of delay in processing after precooking (1927)					
Processed immediately			0	27.3	
Processed after 3 hours			9.1	0	
Processed after 6 hours			18.2	36.4	
Processed after 15 hours			18.2	63.6	
Effect of batch of material					
†Batch canned on 8/23, 1926	36.4	36.4	54.5	100*	
† " " " 8/26, 1926	9.1	0	0	72.7	
† " " " 8/25, 1927	9.1		18.2	18.2	
† " " " 8/26, 1927	18.2		9.1	63.6	
† " " " 9/6, 1927			0	27.3	
† " " " 9/9, 1927	0**		9.1	36.4	
† " " " 9/12, 1927			100	100	81.8

*Percentage based on 10 jars.

**Percentage based on 9 jars.

Other percentages based on 11 jars.

†Noted also in sections above.

TABLE VA. Summary of spoilage of sweet corn canned with batch of material and process time the variables.

Process time in hours	4	3½	3	2	1½
Number jars stored	53	22	77	76	11
Number jars spoiled	8	4	21	45	9
% spoiled	15.1	18.2	27.3	59.2	81.8

The effect of delay in processing after precooking is uncertain. A delay of three hours gave one jar more spoilage in the three hour process, but considerably less in the two hour. A delay of six or fifteen hours resulted in more spoilage in both the three hour and the two hour process.

Effect of addition of acid.—In this series 2.6 c.c. 5 normal phosphoric or 2.0 c.c. 5 normal citric acid was added to 160 grams of cold water poured over the 320 grams of corn for each jar before precooking. The reaction of the water containing phosphoric acid was pH 2.4 and that containing citric

was pH 2.9. The acidified corn after processing was pH 6.0, while that with no acid added was pH 6.9.

The addition of acid had a pronounced beneficial effect on the keeping of the sweet corn. On the day the addition of acid was used as a variable, there was almost 100% spoilage in that canned without acid, while with the acid there was no spoilage in the 2 or the 3 hour process and comparatively little in the 1½ hour. The sweet corn canned with acid had an acid flavor that was strong enough to be objectionable to some individuals. However, when ¼ teaspoon of soda was added to the pint of corn, as it was heated for serving, the acid flavor disappeared and a palatable product resulted. Living organisms which grew and produced acid but not gas in dextrose broth tubes were obtained from 29 of the 33 jars canned without acid on this day, from 4 of the 33 jars canned with phosphoric acid and from 2 of the 33 jars canned using citric acid.

The two hour process time with the addition of either citric or phosphoric acid gave satisfactory results.

Effect of batch of material.—Corn canned on different days showed considerable variation with respect to the proportion which spoiled. Thus of eleven jars prepared on each of a number of days and processed for 3 hours the spoilage records were as follows: Aug. 26, 1927—none; Sept. 6, 1927—none; Aug. 26, 1927—one; Sept. 9, 1927—one; Aug. 26, 1927—two; Aug. 23, 1926—six; Sept. 12, 1927—all. This made a variation of from 0 to 100% in the amount of spoilage using the same method and process time on different days.

The variation in keeping qualities on different days for the various process periods may be summarized as follows:

2	hour process—18 to 100 % spoilage
3	hour process— 0 to 100 % spoilage
3½	hour process— 0 to 36.4% spoilage
4	hour process— 0 to 27.3% spoilage

These results with corn treated as nearly the same as possible showed that an undetermined factor influenced greatly the keeping of the corn. No explanation of the difference in keeping could be gained from a comparison of the maturity of the corn and the resulting difference in consistency of pack and heat penetration, the previous weather conditions, the bacterial count before and after precooking, the pH of the corn, the pH of the water used, the titrable acidity of the corn, the temperature of the ear of corn, or the barometric pressure on the days of canning. Probably the controlling factor is the nature and heat resistance of the organisms that happen to be present on the corn at the time of canning.

Since the difference in spoilage is due to a factor which we did not control and have not definitely determined, we are unable to give data for a completely satisfactory process time for corn without the addition of acid. When the acid was added to the extent of 2.0 to 2.6 c.c. of 5 normal citric or phosphoric acid for each pint of corn, there was no spoilage after two hours processing in the water bath.

SWISS CHARD

Methods used in canning.—The general method used for canning vegetables was used for the chard except that the pans used to precook the chard were large, tightly covered sauce pans. In 1926, only two table-

spoons of water were added to each 475 grams of chard in the precooking. In 1927, $\frac{3}{4}$ cup of water and 350 grams of chard were used for each pint jar. In 1926, the chard was opened after seven months and in 1927 after about five months storage.

The chard that was allowed to stand one day before canning was kept at a temperature of 24° C. in bushel baskets. It was not wilted enough to be noticeable, but had lost 5.5% in weight during storage.

The chard canned with a loose pack contained 204 grams per pint, while that with a solid pack contained 475 grams per pint. Aluminum caps were used on 12 additional pints canned with the loosely packed chard.

The jars canned to show the effect of delay in processing after precooking were allowed to stand in a warm room (24° C.) during the interval between packing and processing. Bacterial counts at intervals, and temperature cooling and re-heating curves were taken. The number of bacteria in the chard after precooking did not increase appreciably in three hours and was only doubled in five hours storage. The chard had cooled to 56° C. at the end of one hour, to 42° C. at the end of two hours and to 36° C. after three hours standing in a room at a temperature of 24° C. It took 45 minutes for the jar that had cooled to 36° C. to reach the processing temperature after it was put into the hot water bath, while the chard which was processed immediately after precooking reached the processing temperature in 15 minutes. This difference was due to the difference in initial temperature in a product with slow heat penetration.

The chard that was canned with the addition of acid contained 1.5 c.c. of 5 normal phosphoric acid or 1.0 c.c. of 5 normal citric acid per 190 c.c. water which was added to each jar. The reaction of the liquid used was pH 3.6 for the citric and pH 3.2 for the phosphoric acid. After processing the reaction of the liquid on the non-acidified chard was pH 6.1, that treated with phosphoric acid was pH 5.8 and that to which citric acid had been added was pH 5.6. After five months' storage the reaction of all jars of chard was about pH 5.4.

Results.—The results obtained from the Swiss chard canning are summarized in Tables VI, VII and VIIA.

Swiss chard canned fresh kept much better than that allowed to stand one day after gathering before canning. This is shown in Table VI.

TABLE VI. Showing the relation of storage before canning to spoilage.

	Canned fresh	Canned after 1 day storage
Processed 2½ hours or more	2.3%*	13.6%
Processed 2 hours	27.3%	45.5%

*Percentages represent spoilage.

In the series of experiments carried out to ascertain the effect of the consistency of the pack, the Swiss chard packed loosely kept better than that packed solidly. There was no spoilage in either pack with the 2½ hour process. For the 2 hour process there was no spoilage in the loosely packed, but 18.2% in the solidly packed jars. For the 1½ hour process the spoilage was 9.1 and 36.4% for the loose and solid packs, respectively. Heat penetration is slow in a substance with the consistency of Swiss chard and the more solid the pack the longer time required for the center of the

jar to reach the processing temperature. This difference in rate of heat penetration into the center of the jar accounts for the greater spoilage in the firmer pack.

The chard canned with aluminum caps kept as well as that for which zinc caps were used.

The chard that was processed at once after precooking showed considerably less spoilage than that in which there was a three or five hour delay in processing. In view of the fact that this delay resulted in a marked drop in temperature (from 87° to 36° C.), the actual period this material which had stood was subjected to the processing temperature was about thirty minutes less than that of those jars which were processed immediately after precooking. This was shown by the heat penetration curves previously mentioned.

TABLE VII. Showing spoilage records of 352 pints of Swiss chard canned under different conditions.

Process time in hours	4	3½	3	2½	2	1½	1
Variable observed	Percentage spoiled						
Effect of storage before canning (1926)							
Chard canned fresh	0	9.1	0	0*	27.3		
Canned after 1 day storage	9.1	18.2	18.2	9.1	45.5		
Effect of consistency of pack (1927)							
Loose pack				†0	0	9.1	
Solid pack				0	18.2	36.4	
Effect of delay in processing after precooking (1927)							
Processed immediately				0		9.1	
Processed after 3 hours				0		54.5	
Processed after 6 hours				18.2		54.5	
Effect of addition of acid (1927)							
No acid added					9.1	18.2	63.6
Phosphoric acid added					0	0	9.1
Citric acid added					9.1	0	0

*Percentage based on 10 jars.

†Percentage based on 23 jars (12 with aluminum caps).

Other percentages are based on 11 jars.

TABLE VIIA. Summary of spoilage of jars of Swiss chard canned under ordinary conditions.*

Process time in hours	4	3½	3	2½	2	1½	1
Number jars stored	11	11	11	55	44	44	11
Number jars spoiled	0	1	0	0	6	8	7
% spoiled	0	9.1	0	0	13.6	18.2	63.6

*Excludes material stored before or after precooking and material to which acid was added.

The addition of acid to the chard increased the probability of its keeping at the shorter process time lengths. In the 1 hour process, 63.6% of the non-acidified chard spoiled, while 9.1% of that with phosphoric acid spoiled and none of that with citric acid. In the 1½ hour process, 18.2% of the "no acid" spoiled and none of that to which either citric or phos-

phoric acid had been added. In the 2 hour process, 9.1% of the "no acid", none of that to which phosphoric acid was added and 9.1% to which citric acid was added spoiled. This 9.1% represented only 1 jar and the absence of spoilage in the 1½ and 1 hour processes with the citric acid series would indicate that this is a chance occurrence and ordinarily we should anticipate no spoilage with the 2 hour process in the presence of the acid.

Of the 55 jars of Swiss chard canned under ordinary conditions, there was no spoilage in the 2½ hour process. The fact that one of the jars processed 3½ hours spoiled makes a spoilage percentage of 1.1 for the 88 jars processed 2½ hours or more. The percentage of spoilage at other process time lengths was 13.6% for the 2 hour, 18.2% for the 1½ hour and 63.6% for the 1 hour. Two and one-half hours was a satisfactory process time for the chard canned.

TOMATOES

Methods used in canning tomatoes.—The tomatoes secured for canning were in poor condition due to a rainy season. When the tomatoes were brought to the laboratory they were sorted and all that showed decomposition were discarded. Thus on the first day only those which showed no decomposing areas were used. Tomatoes to be canned two days later were stored in bushel baskets at a temperature of 21-22° C. After storage, many of the tomatoes had rotted places in them. These were cut out and the tomatoes used. The tomatoes were washed, put into wire baskets, immersed in boiling water for 30 seconds, dipped in cold water and taken out at once. The skins were removed. The tomatoes were cut into quarters and packed tightly into hot jars, and the jars filled to within ¼ inch of the top with tomato juice. The jars were left in hot water (50° C.) for one minute to prevent breakage when plunged into boiling water. The tomatoes canned fresh had a bacterial count of about 500,000 per c.c. of juice as they went into the jar, while those canned after two days storage had a count of over 2,500,000 per c.c.

The storage period for the processed tomatoes was between seven and eight months.

Results.—The condition of the tomatoes when canned made a decided difference in their keeping qualities, as shown in Tables VIII and IX. Tomatoes canned on the day they were gathered showed no spoilage when processed 20 minutes or longer, while those stored for two days before canning showed spoilage in all processes under 35 minutes.

TABLE VIII. Showing the relation of storage before canning to spoilage.

	Canned fresh	Canned after 2 days
Processed 20 minutes or more	0 %*	14.6%
Processed 12 minutes or less	52.4%	42.9%

*Percentages represent spoilage.

The relation of the keeping of the tomatoes to the temperature reached in the center of the jar is shown by the heat penetration figures on tomatoes obtained by Redfield (1927) and noted below.

Length of time of process (in minutes)	Maximum Temperature reached in center of jar— °C.	Number of minutes center of jar was above 60° C.*	Number of minutes center of jar was above 70° C.
5	48.6	0	0
12	57.2	0	0
20	71.1	>35	4
25	72.7	>40	18
35	84.1	>40	33
45	90.5	>55	41

*Taken as an arbitrary temperature for evaluating killing time of non-sporeformers.

It is interesting to note that with the fresh product sterilization was adequate when the process was long enough for the center of the jar to reach 71° C. Because the center temperature continues to rise after the jars are removed from the boiler, the 20 minute process kept the contents at the interior of the jar above 60° C for over 35 minutes.

Twenty minutes was an adequate process time for tomatoes canned shortly after they were gathered, but inadequate for tomatoes that had been kept two days and showed evidence of rot before canning.

TABLE IX. Showing effect of storage of tomatoes before canning on the length of Time Necessary to Process (126 pints).

Process time in minutes	45	35	25	20	12	7
Variable observed	Number spoiled					
Tomatoes canned fresh	0	0*	0	0	6	5*
Tomatoes canned after 2 days	0*	0*	2	4*	1	8*
Percentage spoiled						
Canned fresh	0	0*	0	0	54.5	50*
Canned after 2 days	0*	0*	18.2	40*	9.1	80*

*These figures represent spoilage from 10 jars.

Other figures represent spoilage from 11 jars.

BEEF

Methods used in canning beef.—The beef canned was round and fore-quarter cuts from 20 month heifers which had been killed and kept for one to two days at 0 to 2° C. before processing. Beef was secured from two lots of animals having a similar history, a control series being run each time. The meat was secured from the meat laboratory of the Animal Husbandry Department of the college.

For the control method the meat was wiped with a damp cloth, cut into pieces convenient for packing and 453 gram portions were packed into hot jars. One teaspoon salt was used to each pint jar. The filled jars were placed in hot water for one minute to avoid breakage when plunged into the boiling water.

In one series the relative quantity of water present was used as a variable. Seventy-five c.c. boiling water was added to fill the interspaces in the jars.

To ascertain the effect of the addition of bone in canning beef, a small part of the round bone was allotted to each jar and the weight made up to 453 grams with lean beef.

In another series of observations the effect of the relative amount of fat was studied. This was done by replacing 100 grams of the lean beef by suet in 48 of the jars.

In another series of 48 jars, the beef was partially cooked before being put into the jars. It was weighed, seared in hot frying pans until well browned and packed hot into the hot jars. Seventy-five c.c. of hot water was put into each frying pan and heated to boiling and this unthickened brown sauce was poured over the meat.

Another series was made up of beef that had been allowed to ripen at temperatures from 0-2° C. for 15 days before canning.

The finished jars of beef were stored at about 24° C., observed from time to time and those jars showing evidence of spoilage were removed and examined chemically and bacteriologically. The total period of observation was eight months. At the end of this time the remaining jars were examined.

Results.—No spoilage was shown by the 151 pints of beef processed for 3 or 4 hours. There was 2.6% spoilage in the beef processed 2 hours and 100% of the beef that was processed for 1 hour spoiled. The variables, the presence of bone, the relative amounts of water and of fat, precooking and ripening before canning, made no significant difference in the keeping qualities of the beef. The results obtained with beef are shown in Table X. Three hours was a satisfactory process time for the beef canned under the conditions of the experiments.

TABLE X. Showing spoilage record of 301 pints of beef canned under different conditions.

Process time in hours	4	3	2	1
Material used	Percentage spoiled			
Control beef "A"	0	0	0	100†
Beef with water added	0	0	0	100
Beef with bone added	0	0*	0	100
Beef with fat added	0	0*	10*	100
Control beef "B"	0	0*	0	100*
Beef browned before packing	0	0	8.3**	100
Beef ripened before packing	0	0	0	100*
Summary				
Total jars stored	77	74	77	73
Total jars spoiled	0	0	2	73
Total % spoiled	0	0	2.6	100

*10 jars stored.

**12 jars stored.

†9 jars stored.

Other percentages based on 11 jars stored.

PORK

Methods used.—The variables employed in the canning of pork aside from length of process time were the addition of water, addition of extra fat, precooking before processing and the use of ground loin as compared

with cut pieces of loin. The control method was that used for the beef, 453 grams loin being used for each jar. The loin from 10 months old hogs was used. This was procured one to five days after the hogs had been killed. Loin was secured from two similar lots of hogs, a control series being run each time.

When water was added as a variable 45 c.c. was enough to fill the interspaces of the jar.

The ground loin (sausage) procured was made up of about 70% lean and 30% fat. The amount of sausage used in each jar was 475 grams. The sausage with extra fat added consisted of 425 grams sausage and 50 grams added fat. This made about 37% fat in the sausage.

For the sausage that was partially cooked before packing, 475 grams were weighed and formed into cakes (seven cakes per jar). They were browned well in a frying pan and packed hot into the jars. The fat from the pan was poured over the sausage.

The storage period for the canned pork was over eight months.

Results.—Of the 130 pints of pork processed 3 or 4 hours none showed any evidence of spoilage. These results are summarized in Table XI. Of those given the 2 hour process, 2 of the 10 jars of sausage with extra fat added spoiled. No other spoilage occurred in the 2 hour process. In those jars processed one hour, spoilage was very high, from 45.5 to 100% in all except the sausage that had been cooked before packing. In this series there was no spoilage.

The only variable, aside from time of process, which made a marked difference in the keeping of the product, was cooking of the sausage before packing.

The spoilage in the 2 hour process in the sausage with extra fat added may or may not have been accidental. The heat penetration figures of Redfield (1927) with packs of sausage similar to these showed the sausage with extra fat added took 20 minutes longer to reach the processing temperature than the sausage without the extra fat. This may have been a reason for more spoilage in the series with extra fat. Three hours was a satisfactory process time for the pork canned.

TABLE XI. Showing spoilage records of 262 pints of pork canned under different conditions.

Process time in hours	4	3	2	1
Material used	Percentage spoiled			
Control loin	0	0	0	45.5
Loin with water added	0	0	0	54.5
Control sausage "A"	0*	0	0	100
Sausage with extra fat	0	0	20*	100
Control sausage "B"	0	0	0	100
Sausage browned before packing	0*	0	0	0†
Summary				
Total jars stored	64	66	65	67
Total jars spoiled	0	0	2	44
Total % spoiled	0	0	3.1	65.7

*10 jars stored.

†12 jars stored.

Other percentages based on 11 jars stored.

SUMMARY

1. Spoilage records on 2732 pints of vegetables and meats canned by the hot water bath method are presented.

2. The main variable used throughout the experiments was the length of process time. Other variables for vegetables were, various periods of storage before canning, precooking, scalding or packing cold, addition of phosphoric and citric acids, delay in processing after precooking, character of caps and rubbers, consistency of the pack, lapse of time before completion of the seal, and different storage temperatures after canning. Variables for meats other than process period were the addition of water, of bone, and of fat, partially cooking the meat before processing, the use of ripened beef and the use of ground pork.

3. Process times found entirely satisfactory, under the conditions of the experiments, were $2\frac{1}{2}$ hours for beans and chard, 3 hours for beef and pork, and 20 minutes for tomatoes.

4. A process period of 3 hours for asparagus and 4 hours for sweet corn did not give satisfactory results. When these products were acidified with either phosphoric or citric acid, a process period of 2 hours was found to be sufficient for preservation.

5. Aside from the period of processing, the factors of greatest significance as regards vegetables were length of storage before canning, addition of acid, storage temperature after canning, previous history of the caps employed, consistency of the pack (with chard) and delay in processing after precooking.

6. Precooking the sausage improved the keeping of the product. Other variables studied with meats, aside from processing period, showed no significant differences.

7. Whether better results would be obtained by the home use of the pressure cooker than by using the hot water bath method for the periods found to be satisfactory in this study remains to be established.

8. The work reported in this paper was concerned with the keeping qualities of the processed materials, but the question of the possibility of food poisoning, if the material has been infected with such organisms as *Cl. botulinum*, was not investigated. We are not aware of any evidence, however, of botulinus poisoning from foods canned in Iowa.

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THE INHERITANCE OF RESISTANCE TO THE DANYSZ BACILLUS IN THE RAT¹

M. R. IRWIN²

From the Genetics Laboratory, Iowa State College.

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During recent years, an increasing amount of experimental evidence has added support to the belief that heredity plays an important role in the resistance of the host to an infecting organism. Tyzzer (6) and Hagedoorn (2) have each reported distinct hereditary differences in resistance to natural epidemics among their laboratory mouse stocks. Frateur (1) proposed a single factor pair as governing resistance and susceptibility to avian diphtheria; Roberts and Card (5), and Lambert (3) show results indicating that stocks of poultry more resistant to bacillary white diarrhea and to fowl typhoid, respectively, may be built up by selective breeding, while Webster (7) has demonstrated the possibility of producing stocks of mice more resistant to mouse typhoid infection than a random group of mice not so selected. Pritchett (4) asserts clear-cut differences in susceptibility to mouse typhoid between inbred strains of mice. Wright and Lewis (9) found marked differences in resistance to tuberculosis among inbred families of guinea-pigs.

The experiment of which this paper is a preliminary report was planned to study the influence of heredity on resistance to a bacterial disease by mating survivors of a fixed dose of a specific organism; in short, to produce a highly resistant strain and a highly susceptible strain of rats to a standard dose of the organism.

MATERIALS AND METHODS

The animals used in this experiment came from two distinct sources. The random stock rats (Ra) are descended from two pairs obtained in October, 1925, from Prof. V. E. Nelson of the Chemistry Department, pen inbred by him for six years. In our laboratory, they have been inbred, but not brother-sister. A trio of each of the Wistar "A" and "B" strains was obtained from Dr. Helen Dean King in October, 1924. They have since been maintained by brother-sister matings.

The housing, care, and diet of the animals used in the experiment have been kept as uniform as possible. The age at injection has been approximately 50 days. Naturally, the weight of the animals at this age will vary somewhat, depending in large part upon the number in a litter. Any differences in resistance due to differences in weight will be dealt with in a future report.

¹Paper No. 21 from the Department of Genetics, Iowa State College, Ames, Iowa.

²The author wishes to acknowledge his indebtedness to Dr. E. W. Lindstrom, Dean R. E. Buchanan, Dr. C. H. Werkman, Prof. G. E. Snedecor and A. E. Brandt for many helpful suggestions. Also to the staff of the Department of Bacteriology for valuable cooperation in the use of equipment.

A culture of the Danysz bacillus was obtained in October, 1926, from Dr. R. W. Spray of West Virginia University. This organism had been isolated by him from a commercial rat virus and cultured on plain agar slants. Since arrival at our laboratory, it has been carried on by monthly cultures on veal infusion agar slants.

The pathogenicity of the organism was established at the first injection; after which the procedure outlined below has been followed throughout the experiment.

The media and incubation period of the bacterial culture have been kept constant. Each 18 hr. culture of the Danysz bacillus was washed off in 4 c.c. of sterile physiological salt solution, the suspensions from the different tubes used were mixed in a serum bottle. Since the organism is motile, to facilitate counting it was necessary to heat a 0.5 c.c. sample of the total suspension at 55-60° C. for 20 minutes. Counts were made from the attenuated sample on a Max Levy counting chamber, Helber slide. After the number of organisms per c.c. was determined, the original suspension was diluted to give the desired number of organisms per c.c. Each animal was injected intraperitoneally with 0.25 c.c. of the determined suspension. The deaths for the preceding 24 hours were recorded each morning at about 10 o'clock. In general, only those deaths were recorded which occurred between the 2nd and 14th days.

Since considerable time elapses after making the suspension of bacterial cultures in physiological salt solution until counting and injecting are completed, the number of organisms injected may vary somewhat from that determined in counting. Tests are in progress to determine the viability of the Danysz bacillus in saline solution. Winslow and Brooks (8) report marked differences in viability between different types of bacteria in suspension in distilled water and likewise in saline solution. However, the time between making the suspension, counting the organisms, and injecting the animals has been relatively constant throughout the experiment. Any change, then, in the number of organisms in the suspension will be in a constant relative ratio to the number injected.

The virulence of the organism has remained practically constant, judging from the results obtained by injecting the same number of organisms into control stocks from month to month. The writer has noticed no results that would indicate a change in the virulence since the beginning of the experiment.

Determination of the Standard Dose

As a preliminary step, it was deemed necessary to determine the effect of different numbers of organisms upon the host. A dose sufficient to cause the death of approximately 85% of the animals tested was the goal desired. This number, when determined, was to be used as a minimum for further work, and has been termed the "standard dose", as opposed to the "minimum lethal dose". The results of these preliminary trials to fix the standard dose are shown in Table I.

The results of these preliminary tests with different numbers of the Danysz bacillus indicate a close correlation between decrease in dose and decrease in mortality. As the number of bacteria increases, the effect as shown by the mortality becomes less; seemingly, then, individuals resistant to a relatively large number of organisms are affected only slightly by a further increase in the number of bacilli injected.

TABLE I. Percentage mortality in rats due to varying doses of the Danysz bacillus.

Dose (Millions)	Number Tested	Number Dead	Percentage Mortality
4000 +	70	69	98.5
2-4000	39	39	100.0
275	104	98	94.2
210	93	85	91.3
150	168	139	82.7
120	72	46	63.9

A mathematical analysis of the differences in percentage of mortality due to successive, varying numbers of organisms gives no certainty that factors other than random sampling are the cause. The table, however, is a summary of tests over a period of a few months, and gives the average for the tests of each dose. It may be added that the range for the percentage mortality of each dose was quite narrow. For example, the dose of 150×10^6 organisms produced an average percentage mortality of 82.7. The range for all percentage mortalities produced by this dose is from 77.5 to 86.3; one-half the results lie within the limits of 80.4 to 85.0.

This number of organisms (150×10^6) was chosen as the standard dose and has since been used as the minimum number of organisms to be injected.

Effect of Selection

TABLE II. Percentage mortalities of stocks of rats following injection of the standard dose of the Danysz bacillus.

Stocks	Number Injected	Number Dead	Percentage Mortality
Wistar "A" strain	96	30	93.7
Wistar "B" strain	31	25	80.6
Random Stock (Ra)	168	139	82.7
R ₁ stock	123	48	39.0
R ₂ stock	71	17	23.9
R ₁ A stock	147	69	46.9

In the above table, the nomenclature of the different stocks is as follows: the Wistar "A" and "B" strains, and the random stock (Ra), are our regular laboratory stock rats. The progenies of Ra survivors of the standard dose of the Danysz bacillus represent the first resistant generation, R₁; likewise R₂ denotes the second resistant generation. Individuals of the R₁ stock surviving the standard dose were mated to non-tested "A" strain males and females; the progeny are termed R₁A stock. Until October, 1927, the Ra stock has been used as control stock in the experiment; since that date, the "A" strain has served as controls at the different injections.

It will be noted in Table II that the Wistar "A" strain was somewhat more susceptible to the standard dose than the Wistar "B" strain. However, the probability ($P = .64$) that this difference was due to other than random sampling is slight, although there are other factors which would seem to indicate that something other than chance has determined the difference. A uniformly higher percentage of deaths of the "A" strain which has occurred in a series of injections tends to strengthen the

TABLE III. Mathematical analysis¹ of differences in percentage mortality between stocks of rats shown in Table II.

Stocks compared	Differences in Percentage Mortality	X ²	P
"A" and "B"	13.1	0.24	0.64
R ₁ and Ra	43.7	13.64	*
R ₁ and R ₂	15.1	2.31	0.13
R ₁ and "A"	54.7	15.52	*
R ₁ A and "A"	46.8	11.31	*

*Probability very great that the differences are due to causes other than chance ($P < 0.01$).

¹The calculations have been made according to Fisher's "Contingency Tables". Fisher, R. A. 1925. Statistical Methods for Research Workers, p. 84. Oliver and Boyd, London.

belief that a significant difference would appear if a larger number could be used. Hence the "A" strain has been selected as a more susceptible strain for purposes which will be dealt with a little later in this paper.

There was practically no difference between the "B" strain and Ra stocks in their reaction to the standard dose. In this report, the survivors of the standard dose giving rise to the R₁ stock have been confined to the Ra stock. The first resistant generation of the inbred "B" strain (R₁ "B" strain) is being developed at present; but the results as yet are in insufficient numbers to be included in this paper.

The R₁ stock showed a decided increase over the control stock injected at the same time in the number of animals surviving injections of the standard dose. A comparison of the difference in resistance between the R₁ stock and the average of the parental Ra stock gives a value for X² of 13.6. Here it is certain that factors other than chance have operated in determining the difference in resistance.

Likewise, the R₂ stock in turn gave an average of more individuals resistant to the standard dose than the average of the R₁ stock. The difference between these two stocks approaches significance ($P = .13$) and may be interpreted as having been due in large part to selection within the R₁ stock.

Previous to the testing of the R₂ generation, the R₁ stock and the "A" strain differed most widely in resistance. It has been stated before that the "A" strain is considered as the "susceptible" strain. The reaction of the progeny from matings between these R₁ and "A" stocks has been of value from two distinct points of view; it has afforded an opportunity to study the method of inheritance of the resistance, and to determine the effect of any passive immunity that might have been transmitted by the survivors to their offspring.

The increase in percentage of individuals of this progeny, R₁A stock which survived the standard dose, is significant when compared with the mortality of the "susceptible" "A" strain parent. The odds are extremely great that the increase in resistance of this stock was in large part due to the influence of the R₁ parent, since X² for this difference equals 11.31.

The resistance was contributed to this progeny by either R₁ parent as shown below:

Parents	Number Tested	Number Dead	Percentage Mortality
$R_1 \sigma \sigma \times A \varphi \varphi$	85	38	44.7
$R_1 \varphi \varphi \times A \sigma \sigma$	62	31	50.0

It will be noted that there was an approximate equality of the R_1A stock, when the R_1 parent was either male or female. The seeming difference in mortality between these progenies (44.7-50.0) was largely due to the difference in mortality between sexes in this particular progeny. An analysis of this difference is shown as follows:

Mating	Sex	Number Tested	Percentage Mortality
$R_1 \sigma \sigma \times "A" \varphi \varphi$	$\sigma \sigma$	46	39.1
$R_1 \sigma \sigma \times "A" \varphi \varphi$	$\varphi \varphi$	39	51.2
$R_1 \varphi \varphi \times "A" \sigma \sigma$	$\sigma \sigma$	27	44.4
$R_1 \varphi \varphi \times "A" \sigma \sigma$	$\varphi \varphi$	35	54.2
Total	$\sigma \sigma$	73	41.0
Total	$\varphi \varphi$	74	52.7

There was a somewhat higher number of deaths among the females in this progeny than among the males. Since there was a larger number of females in proportion to the males in the offspring of the cross, $R_1 \varphi \varphi \times "A" \sigma \sigma$, the percentage mortality of this offspring would necessarily be increased slightly as compared with the reciprocal cross ($R_1 \sigma \sigma \times "A" \varphi \varphi$), in which the males were in a slight majority.

Were the increased resistance of the R_1 and R_2 generations over the R_a stock due in part to the transmission of passive immunity, the offspring of the mating $R_1 \varphi \varphi \times "A" \sigma \sigma$ should have shown a higher degree of resistance than those from the reciprocal cross ($R_1 \sigma \sigma \times "A" \varphi \varphi$) due to the influence of the dam. There is no case reported in the literature within the knowledge of the author in which an immunized male has transmitted passive immunity to the offspring. Hence, the effect of passive immunity as a factor in increased resistance of selected stocks in this experiment, is at a minimum, since the progeny (R_1A) from the cross, $R_1 \times "A"$, has shown no difference in effect from either parent being resistant.

SUMMARY

The number of organisms of the Danysez bacillus that was injected determined within reasonable limits the percentage mortality of a random sample of rats.

Slight differences in resistance to the standard dose of the Danysez bacillus have been found between two inbred strains of rats, Wistar "A" and "B". In a random stock of rats progeny from matings of survivors of injection of a fixed number of this organism, were more resistant to the same dose than a random group of the same stock.

Offspring of a cross between the first resistant generation and the susceptible "A" strain were much more resistant to the disease than the susceptible parent. There was no evidence that this increase in resistance was due even in part to the transmission of passive immunity.

It is suggested from these results that resistance in this case is due to a partially dominant, quite complex set of factors, whose interactions with the environmental factors determine the reaction of the individual to the infection.

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SPECIES CROSSES IN THE GENUS CUCURBITA

By EDWARD F. CASTETTER

From the laboratories of the Department of Botany, Iowa State College.

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INTRODUCTION

A perusal of the literature dealing with interspecific hybrids in *Cucurbita* leaves considerable uncertainty as to the limits of intercrossing in this genus. Certain of the investigators in this field were wholly unable to secure hybrids between the species, whereas others achieved positive results; the present work was undertaken, therefore, with the hope of throwing additional light on the problem.

Five of the ten species comprising this genus have been employed by previous investigators in their hybridization experiments. In the writer's investigations three species were used—*C. pepo*, *C. maxima*, and *C. moschata*, these being the only annual species in the genus, as well as the only species cultivated in the United States.

The results given herein represent the work of six consecutive years carried on in both field and greenhouse in an effort to secure hybrids among the three above mentioned species.

HISTORICAL

As early as 1854 Naudin (10) attempted to intercross various species of *Cucurbita*, viz: *C. maxima*, *C. pepo*, *C. moschata*, *C. melanosperma* and *C. perennis*. The result of a comparatively small number of controlled pollinations was a few fruits, none of which contained fertile seeds.

It was more than thirty years later that a similar line of investigation was undertaken by Bailey (1, 2). He worked for ten consecutive years cross-pollinating varieties, species and genera of the Cucurbitaceae. As a result of numerous efforts with the three cultivated species of *Cucurbita*, seven fruits were secured: two by pollinating *C. pepo* x *C. maxima*, one by *C. moschata* x *C. maxima*, one by *C. maxima* x *C. pepo*, and three by *C. pepo* x *C. moschata*. However, fertile seeds were found only in the three fruits obtained from *C. pepo* x *C. moschata*, and eighty-eight F_1 plants were grown from the seeds in the two fruits resulting from Connecticut Field Pumpkin x Japanese Crookneck. (No mention is made of the seeds from the third fruit—Gourd x Japanese Field Pumpkin.) A number of F_2 plants were also grown and one fruit secured, but Bailey does not state whether this fruit contained fertile seeds. Somewhat at variance with the findings of Bailey are those of Pammel (11), who concluded it is impossible to obtain hybrids between the different species of *Cucurbita*.

Additional data have been furnished by Drude (4, 5), whose researches extended over a period of twenty-five years. Only three fruits were produced and these by the pollination of *C. pepo* (white apple) with *C. ficifolia* (*C. melanosperma*). Two of these fruits were entirely sterile, the third contained one fertile seed. This hybrid was in turn successfully crossed with a Fordhook (*C. pepo*). Drude's attempts between 1901 and 1905 to repeat this cross between *C. pepo* and *C. ficifolia* failed, as did all attempts

to cross other species of the genus during his twenty-five years of experimentation.

The work of the Hagedoorns (6, 7) is of considerable interest in that they not only secured several interspecific hybrids (*C. maxima* x *C. pepo*), but also apparently found that some of the F_1 hybrid plants set fruit and produced viable seeds from unpollinated female buds. Only three F_1 interspecific and two F_1 intervariety hybrids developed parthenogenetic seeds. The F_2 and F_3 generations in each case were grown for the purpose of ascertaining whether true parthenogenesis or merely apogamy was indicated. By this genetic method it was found that in at least four of the five cases the progenies gave strong evidence of parthenogenesis. That parthenogenesis in *Cucurbita* is closely associated with hybridity finds support in the fact that 106 female flowers of 18 varieties, carefully protected from pollination, failed to produce a single seed. It is worthy of note that the three fruits resulting from the pollination of *C. maxima* (Turkenbund) x *C. pepo* developed on a single Turkenbund plant. Their efforts to intercross species other than *C. maxima* x *C. pepo* were unsuccessful.

In discussing the investigations carried on by the Hagedoorns, Lotsy (8, 9) thinks the possibility of crossing *C. maxima* with *C. pepo* has not been proved. Although he made unsuccessful attempts to cross these species himself, he feels his work has not been sufficiently intensive to definitely conclude that crosses cannot be made between *C. maxima* and *C. pepo*. He is convinced, however, from his own investigations that neither *C. pepo* nor *C. maxima* can be crossed with *C. melanosperma*. Lotsy's results do not confirm those of the Hagedoorns regarding the occurrence of parthenogenesis in unpollinated F_1 hybrids.

The most recent work with a view to crossing the species of *Cucurbita* is that of Vavilov (12), who made reciprocal pollinations among four species—*C. pepo*, *C. maxima*, *C. moschata* and *C. melanosperma*. Not only was he unsuccessful in obtaining hybrid seed, but in not a single case was fruit secured as a result of his pollinations. He, too, was unable to confirm the phenomenon of parthenogenesis as reported by the Hagedoorns. Vavilov cites the work of Miss Koslov (unpublished), at the Turkistan Agricultural Experiment Station, who secured four hybrid seeds by pollinating *C. maxima* with *C. moschata*. The F_1 fruits were entirely without fertile seeds, as Vavilov personally observed in December, 1924. His conclusion is: "Species of *Cucurbitaceae* are so different that to get fertile hybrids among them, and especially among different species of *Cucurbita*, is impossible."

Summarizing the results reported in the above literature, the interspecific hybrids obtained were a considerable number of fertile F_1 hybrids of *C. pepo* x *C. moschata*, by Bailey; one F_1 hybrid plant of *C. pepo* x *C. ficifolia* (*C. melanosperma*), by Drude; a number of F_1 hybrids of *C. maxima* x *C. pepo* by the Hagedoorns, and four sterile hybrids of *C. maxima* x *C. moschata*, by Miss Koslov (unpublished).

MATERIAL AND METHOD

The investigations were carried on with two general types of seeds—pure lines, and that obtained from numerous commercial seed houses throughout the United States. In all cases numbers have been used to designate the pure lines, while variety names have been employed for commercial forms,

The sources of pure lines are:

Line No. 175—Connecticut Field Pumpkin (*C. pepo*) developed by the writer by inbreeding.

Lines No. 48a, b, c—Patty Pan, or Scallop, Pumpkin (*C. pepo*), from Dr. E. W. Sinnott, of the Connecticut Agricultural College.

Line No. 270—Hubbard Squash (*C. maxima*) from M. B. Cummings, of the Vermont Agricultural Experiment Station.

Line No. 20—Hubbard Squash (*C. maxima*) from John W. Bushnell, then of the Minnesota Agricultural Experiment Station.

Line No. 5—Large Cheese, or Kentucky Field, Pumpkin (*C. moschata*), developed by the writer by inbreeding.

Plants were all grown on the grounds or in the greenhouse of the Iowa Agricultural Experiment Station. The work consisted of cross pollinating varieties between the three species, and since descriptions of these species are given in an earlier paper by Castetter and Erwin³, these will not be included here. As Cucurbita is monocious and has very large flowers it was easy to prevent contamination by foreign pollen. The method used was to isolate both staminate and pistillate flower buds by tying the corolla with a heavy cord on the evening previous to opening. On the following morning the cord was removed from each flower and the stamens from the desired variety rubbed over the stigma of the pistillate flower, which was again tied with the cord, properly tagged and labelled—the various types of pollinations being designated by tags of different colors attached to the fruit stalk. Especial care was exercised in tying the buds and flowers to preclude the possibility of pollination by insects; also flowers torn in handling, or those concerning which there was any doubt as to complete isolation, were discarded. Immediately before being removed from the vines all fruits were tagged a second time to prevent confusion of identity in handling. Fruits were allowed to remain unopened for a month or more after being removed from the field or greenhouse, in order to allow seeds to fully mature.

RESULTS

Each series of pollinations, with the fruits and seeds resulting therefrom, is given in condensed tabular form according to species, and is a summary of investigations carried on in both field and greenhouse over a period of six years.

C. PEPO x *C. MAXIMA*

Parents	Number Pollinations	Fruits	Fertile Seeds
Connecticut Field x Hubbard	93	10	0
175 x 270	51	4	0
175 x 20	70	4	0
Connecticut Field x Marblehead	152	12	0
Connecticut Field x Victor	120	6	2
48a x 20	54	17	0
48b x 20	74	8	0
48c x 20	53	17	6
	<hr/> 667	<hr/> 78	<hr/> 8

From the above table it will be seen a number of parthenocarpic fruits were secured. In neither successful cross, however, were the fertile seeds all found in a single fruit. Of the two obtained from Connecticut Field x Victor, one seed was found in each of two fruits. The six seeds resulting from the 48c x 20 cross were distributed among four fruits. These eight hybrid seeds appeared in fruits grown in the field during the summer of 1927. At present the eight F_1 hybrid plants are vigorously growing in the greenhouse, but are too young to make their description of any value.

C. MAXIMA x *C. PEPO*

Parents	Number Pollinations	Fruits	Fertile Seeds
Hubbard x Connecticut Field	176	22	0
270 x 175	285	13	0
20 x 175	434	38	11
Marblehead x Connecticut Field	142	14	0
Victor x Connecticut Field	38	7	0
Delicious x Connecticut Field	57	5	0
20 unpollinated (flowers tied without pollinating)	43	0	0
	<hr/> 1175	<hr/> 99	<hr/> 11

These eleven 20 x 175 hybrid seeds appeared:

In the field, 1924, 4 seeds in 1 fruit
1925, 3 seeds in 2 fruits
1926, 2 seeds in 2 fruits

In the greenhouse, 1924, 2 seeds in 2 fruits.

The resulting F_1 hybrid plants were all grown in the greenhouse.

No. 20, a pure line of Hubbard squash and the pistillate parent of the hybrids, is characterized by rough heavy stems, leaf blades and leaf stalks; rather kidney shaped leaves with rounded lobes and indistinct sinuses between the lobes. The fruit stalk is cylindrical and spongy. Shape of the fruit nearly cylindrical, but pointed at blossom end. Surface bumpy, color glossy dark green. Shell hard at maturity. Size 9 x 6 inches, weight about 6 pounds.

No. 175, a pure line of Connecticut Field Pumpkin—the staminate parent of the hybrids—has spiny stems, leaf blades and leaf stalks; the leaves are strongly lobed and have deep sinuses between the lobes. Fruit stalk hard at maturity, five-sided, distinctly grooved, not noticeably enlarged at attachment to fruit. Fruit round, flattened at both ends. Surface smooth, color orange yellow, shell thin and hard. Size about 16 x 10 inches, weight about 20 pounds.

From the eleven F_1 hybrid seeds of 20 x 175 only four plants grew to maturity, seven of the seeds giving rise to feeble plants which died when from four to six inches tall. These four plants were the progenies of two 20 x 175 fruits, each from a different plant; three of the seeds having come from one fruit and the fourth seed from another. The single hybrid plant

from the first fruit has been designated as No. 100 and the three hybrid plants from the second fruit as No. 101.

Three cuttings were made from No. 100 and the three resulting plants were grown in the greenhouse. No. 100 is characterized by prickly stems, leaf blades and leaf stalks. Leaf blades distinctly lobed with deep sinuses between the lobes. It will be noted that these vegetative characteristics are very similar to those of the staminate parent, No. 175. These plants bore very few flowers, either staminate or pistillate. All the pollen was abortive, and examination with the microscope showed it to be badly shriveled. Although self pollination of No. 100 resulted in no fruits, several fruits were secured by back crossing, as will be seen from the following table. These fruits were nearly spherical in shape, varied greatly in size, had hard, thick, warty shells and in color were dark green with a faint gray mottling. The seed coats and fruit stalk closely resembled those of *C. pepo* (175). None of these fruits contained any fertile seeds.

The above description of No. 100 will also serve for No. 101, with the exceptions that the terminal lobe of the No. 101 leaf was much shorter than that of No. 100, and the fruit of No. 101 was light green, decidedly mottled with gray and yellow, as opposed to the dark green and faint gray mottling of No. 100. The three No. 101 plants were very similar to each other in all respects.

POLLINATION OF F_1 HYBRIDS OF *C. MAXIMA* \times *C. PEPO*

Parents	Number Pollinations	Fruits	Fertile Seeds
100 Selfed	6	0	0
100 Open pollinated in field		3	0
100 Unpollinated (flowers tied without pollinating)	12	0	0
100 \times 175	6	2	0
100 \times 20	3	1	0
20 \times 100	5	0	0
101 Selfed	9	0	0

The small number of pollinations recorded for this first hybrid generation is due to the few flowers formed on these plants.

C. PEPO \times *C. MOSCHATA*

Parents	Number Pollinations	Fruits	Fertile Seeds
175 \times 5	134	14	37
Conn. Field \times Striped Cushaw	24	1	Many
Early White Bush Scallop \times Large Cheese	3	1	0

The pistillate parent in the 175 \times 5 cross has been described earlier in the paper. No. 5, the staminate parent, has soft hairy stems and leaves. The leaf blades are weakly lobed without notches between the lobes, and have silvery spots at intersections of veins. Fruit is round, flattened at both ends, giving the appearance of a cheesebox. Shell thin, but hard,

smooth. Colory, creamy buff. Fruit stalk hard, five-sided, deeply grooved and distinctly flaring at attachment to fruit.

The hybrid seeds obtained from Conn. Field x Striped Cushaw were planted in the greenhouse and gave rise to vigorous plants, which are not yet sufficiently mature for satisfactory description.

The F_1 plants of 175 x 5 (designated as No. 125) were grown in both greenhouse and field. The shape of the leaf and the degree of prickliness of leaf and stem were intermediate between those of the parents. The fruit shape is short oblong, and the color a network of green over a light orange background. The network, incompletely covering the background, gives to the fruit the appearance of being splotched. These fruit characteristics are difficult to understand when we consider the shape and color of both parents. The shell of fruits is hard, smooth or slightly warted. Fruit stalk is flaring at attachment as in the staminate parent. Self pollinating No. 125 shows this F_1 generation to be very fertile, as many viable seeds were secured out of the 12 fruits resulting from 19 pollinations.

From these seeds a large number of F_2 plants were grown. Since it was not primarily the purpose to report the inheritance of characters in these crosses, no details will be given on the F_2 generation except that it was somewhat fertile, for out of 62 self pollinations of the F_2 (designated as No. 127) 19 fruits with many viable seeds were secured.

C. MOSCHATA x *C. PEPO*

Parents	Number Pollinations	Fruits	Fertile Seeds
Large Cheese x Conn. Field	294	18	0
Striped Cushaw x Connecticut Field ...	62	18	0
5 x 175	31	1	0
	<hr/> 367	<hr/> 37	<hr/> 0

The failure to secure any fertile seeds of *C. moschata* x *C. pepo* is very surprising, in view of the large number of fertile F_1 , also fertile F_2 , seeds secured in the reciprocal pollinations.

C. MAXIMA x *C. MOSCHATA*

Parents	Number Pollinations	Fruits	Fertile Seeds
Hubbard x Large Cheese	3	1	0
270 x 5	2	1	Many
20 x 5	62	23	Very many

Pollination of F_1 hybrids

15 Selfed	2	0	0
15 Unpollinated (tied up without pollinating)	63	0	0
15 x 20	87	18	Few
15 x 5	103	28	Few
20 x 15	13	2	0
5 x 15	5	0	0

Of all attempts to cross species, *C. maxima* x *C. moschata* proved to be the least difficult, it being possible to secure hybrids at will. The F₁ plants of 20 x 5, (designated No. 15), showed remarkable hybrid vigor. The stems and leaves were rough hairy, leaf blades intermediate in shape between those of No. 20 and No. 5, with silvery spots at intersections of veins. Pistillate flowers abundant, staminate flowers very few and sterile. Numerous fruits were secured, however, by back crossing and open pollination. The fruits were nearly spherical in shape. Shell thick, hard, bumpy, of uniform dark green color. Fruit stalk hard, five-sided, grooved, but not flaring at attachment.

No. 18, secured by pollinating 15 x 20, and No. 19, resulting from 15 x 5—both back crosses—were grown in the field. It is apart from the pupose, however, to give details concerning the characteristics of these back crosses.

C. MOSCHATA x *C. MAXIMA*

Parents	Number Pollinations	Fruits	Fertile Seeds
5 x 20	8	2	0
Large Cheese x Hubbard	192	41	Many
Striped Cushaw x Victor	1	1	0
Striped Cushaw x Marblehead	48	25	1
	<hr/> 249	<hr/> 69	<hr/> Many

The small number of pollinations of the pure lines 5 x 20 is due to No. 5 flowering very late in the season—too late to make pollination worth while.

The single seed resulting from Striped Cushaw x Marblehead produced a plant which is now growing in the greenhouse but is not sufficiently mature to make determination of definite characteristics possible. The hybrid seeds resulting from Large Cheese x Hubbard have not yet been grown.

DISCUSSION

In planning the above investigations considerable thought was given the method to be used in protecting the flowers from contamination by foreign pollen. Several investigators in the field have isolated by tying a string or wrapping soft wire around the tips of the buds; others have made use of paper or parchment bags for this purpose. Both Lotsy (8, 9) and Vavilov (12) have criticized the former method, maintaining that it does not entirely exclude the possibility of the entrance of insects. The writer's experience has been that both methods are equally unsafe in careless or inexperienced hands, and that perfect isolation is secured by tying the corollas if proper care is exercised. The writer chose to protect against contamination by tying the corolla tips, rather than by the paper bag method, as the exclusion of light and interference with the circulation of air in the latter method are very possible limiting factors in crossing species, even though this is not the case in crossing varieties. In this connection it is worthy of note that both Lotsy and Vavilov are the only work-

ers who report having used the paper bag method, and neither secured hybrid seeds.

The phenomenon of parthenogenesis reported by the Hagedoorns (6), in which they secured fertile seeds from unpollinated F_1 interspecific and intervariety hybrids, has been reinvestigated by Lotsy. He covered a considerable number of unpollinated female flowers on intervariety hybrid plants with paper sacks, but in not a single case did he obtain seeds.

Vavilov enclosed in parchment bags a number of female flowers of various genera and species of the Cucurbitaceae, but was unable to secure parthenogenetic seeds. It should be noted, however, that his work differed from that of the Hagedoorns in that he did not use hybrid plants. The writer made no systematic effort to study parthenogenesis in Cucurbita, but some data have been accumulated. With No. 20—a pure line of Hubbard squash—43 female flowers were tied without being pollinated. No fruits were obtained. In No. 100—the F_1 hybrid of 20×175 —12 unpollinated female flowers were tied, but no fruits developed. Sixty-three unpollinated female flowers of No. 15—the F_1 hybrid of 20×5 —were isolated in a similar manner, but no fruits resulted. While these data are quite meager, nevertheless so far as they go they fail to confirm the results of the Hagedoorns.

While a considerable number of hybrid seeds were secured in the above investigations, it is evident many of the pollinations produced nothing more than parthenocarpic fruits. This is in harmony with the results obtained by other workers in the field. It was observed, however, that when such parthenocarpic fruits were stored they decayed much more quickly than did fruits containing fertile seeds stored under identical conditions. An examination of the interior of parthenocarpic fruits invariably revealed the presence of many well formed seed coats which were either collapsed or inflated, and without embryos; in some cases, however, small, partially developed and undifferentiated embryos were observed, although these were too rudimentary to produce plants.

CONCLUSIONS

1. The literature dealing with interspecific hybrids in the genus *Cucurbita* is reviewed and summarized.

2. A large number of cross pollinations between the three annual cultivated species of *Cucurbita*—*C. pepo*, *C. maxima* and *C. moschata*—with the results obtained therefrom, are reported. Both pure lines and commercial varieties were used in the investigation.

3. Using *C. pepo* as the pistillate and *C. maxima* as the staminate parent, eight hybrid plants were secured, the fertility of which has not yet been investigated. Reciprocally, the result was eleven fertile hybrid seeds, all the F_1 plants of which were entirely self sterile. Fruits developed on these plants only as the result of back crossing and open pollination, and in no

4. *C. pepo* \times *C. moschata* gave rise to many seeds; both the F_1 and F_2 were quite fertile, a considerable number of viable F_3 seeds resulting. *C.*

case did a fruit contain viable seeds.

Moschata \times *C. pepo*, however, yielded no viable seeds.

5. No difficulty was experienced in crossing *C. maxima* with *C. moschata*. Without exception the F_1 was self sterile on account of abortive pollen. But it was found possible to procure fruit containing viable seed

from the F_1 plants by back crossing. Reciprocally, more effort was required, although the hybrids were fairly numerous. The fertility of the F_1 has not yet been studied.

6. Parthenocarpy was observed to be quite common, but in no instance was parthenogenesis observed.

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ARITHMETICAL CHANGES IN STATISTICAL CONSTANTS DUE TO CODING AND THEIR CORRECTION

By A. E. BRANDT.

From the Department of Mathematics, Iowa State College.

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The process of coding as presented herein is a process by which, for expediting the calculation of statistical constants, more or less fictitious values are substituted for the observations without sacrificing their identity. It is the equivalent of the usual grouping into classes and translating the origin of measurements, which process, under the head of grouping, has been discussed by many writers on statistics including G. Udny Yule (1916), Truman L. Kelley (1923), Horace Secrist (1925), and R. A. Fisher (1925).

It seems to be rather universally agreed that grouping is a legitimate process, at least so far as the frequency distribution is concerned, but there is no agreement as to the exact process of grouping. The number of groups or class intervals and the class limits are doubtful points. Yule (1916) says that the number of classes should lie between 15 and 25 and that less than 10 classes leads to very appreciable inaccuracy; Kelley (1923) says that there should not be less than 12 classes; while Fisher (1925) says that the class interval should not be less than one-fourth the standard deviation, which is the equivalent of saying that there should be not less than 24 classes, since the total range of a variable is very approximately six times the standard deviation. Secrist (1925) says on page 168, "In writing the limits of groups, a smaller fraction of the whole unit should not be used than was employed in the actual process of measurement." Whereas Yule (1916) says on page 81, "The difficulty may always be avoided if it be borne in mind in fixing the limits to class intervals, these being carried to a further place of decimals, or a smaller fraction, than the values in the original record."

Before entering upon the problem illustrating the arithmetical changes in the various statistical constants, we will present the method of coding used. Only continuous series or discrete series in which the range is large in respect to the unit of measurement should be coded. Anyone may easily code a series by complying with the following five rules and one caution:

1. Determine the actual range of the series from the data and decide the number of class intervals into which the series is to be divided.
2. Divide the actual range by the number of classes and select a convenient class interval larger than the quotient.
3. Multiply this selected class interval by the number of classes and from this theoretical range subtract the actual range, take one-half of this difference, and subtract it from the least observation to secure the lower limit of the first class interval.

4. Add one-half of the selected class interval to the lower limit of the first class for the first class mark.

5. Set up the coding equation as follows:

$$\text{Observation (coded)} = \frac{\text{Observation} - \text{First class mark}}{\text{Class interval}}$$

Caution: Examine the coded series carefully to make sure that if there is a clustering of observations it will be about the mid-point or center of the class interval and not at the limits. If there is a clustering at the limits, the coding must be done over with a different class interval because the mid-point or class mark is substituted for the actual values, thus introducing inaccuracies which are inversely proportional to the density of grouping about the class mark.

The set of data used in this illustration consists of the total intelligence test score (I), an individual intelligence test score (G), total high school average (H), high school average in one subject (R), and first quarter college grade (Q), of 492 Iowa State College freshmen. Test G is a part of test I and average R is a part of average H. These were selected in this manner so as to insure some high correlations. The first quarter college grade (Q) is the dependent variable.

Each variable was coded into 10 classes and then into 20 classes. The coded values and the original observed values were punched on data cards for use in sorting and tabulating machines as shown in Plate I. With the aid of the sorting and tabulating machines and of calculating machines, the means, standard deviations, simple correlation coefficients, and multiple correlation coefficient were calculated following the tabular form presented by H. A. Wallace and George W. Snedecor (1925). The means and standard deviations of the coded sets are expressed in the code units and must be decoded, that is, expressed in the original units. In this illustration the means are adjusted by multiplying by the class interval and adding the first class mark. The standard deviations are adjusted by multiplying by the class interval. The decoded values of the constants and their probable errors are given in Table I.

Assuming that the values of the constants obtained from the uncoded data are correct, the values obtained from the coded sets differ from them as was expected. In this example, the differences are not great enough to cause any serious difference in interpretation, but the fact that they occur warrants investigation as to the possibility of correction. The correction to be used is, of course, the commonly used Sheppard's correction. Concerning the advisability of using Sheppard's correction, Fisher (1925) on page 150 says, "... , and the full effects on the distribution in random samples of using Sheppard's correction have never been fully examined, but there can be little doubt that Sheppard's correction should be used, and that its use gives generally an improved estimate of the correlation."

The tabular calculation of the correlation coefficients given by Wallace and Snedecor (1925) makes it possible to use Sheppard's correction with only a minor change. Five lines are provided for each variable instead of four. The first column appears as follows:

Sums	ΣA
Means	M_A
A_1	ΣA^2
A_2	$(\Sigma A) M_A$
A_3	$\Sigma A^2 - (\Sigma A) M_A$
A_4	$\Sigma A^2 - (\Sigma A) M_A - n/12$
A_5	$\sqrt{\Sigma A^2 - (\Sigma A) M_A - n/12}$

The leading entry in each block of lines is made to comply with the one above, the entry in line 4 being the usual entry in line 3 diminished by Sheppard's correction $n/12$. The subsequent entries in any block of lines is exactly as given by Wallace and Snedecor (1925) except that line 5 provides a place to enter the simple correlation coefficients. It is evident from the above illustration that Sheppard's correction does not affect the mean.

Those constants that are affected by Sheppard's correction are given in Table II in decoded form. The values in the column headed uncoded are the same as those in the same column in Table I, but are repeated here for comparison. From this table we find that Sheppard's correction has adjusted the value of each constant so that it lies within the probable error of the value secured from the uncoded set except the standard deviation of I (σ_1). This discrepancy is probably due to the wide range of I (from 57 to 277).

It appears from the above results that it is advisable to use Sheppard's correction if we desire to get the estimate which most closely approximates the value secured from the uncoded data. On the other hand, if we take the more usual statistical view that the present set of data is merely a random sample from a population and that the values secured for the various constants are approximations to the real values, the arithmetical changes due to coding are not large compared to the errors of random sampling so that the use of Sheppard's correction is not imperative. In fact, Sheppard's correction should seldom be used with small samples. As Fisher (1925) says on page 150, "The fact that with small samples the correlation obtained by the use of Sheppard's correction may exceed unity, illustrates the disturbance introduced into the random sampling distribution."

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TABLE I. The Statistical Constants and Their Probable Errors Without Correction.

Constant	Coded		Uncoded
	10 classes	20 classes	
M_I	142.2680 ± 1.0573	141.8265 ± 1.0397	141.5183 ± 0.8895
M_G	17.3488 ± 0.1337	17.2238 ± 0.1276	17.2093 ± 0.1299
M_H	86.9818 ± 0.1416	86.5651 ± 0.1427	86.4370 ± 0.1412
M_R	86.2276 ± 0.1652	86.2276 ± 0.1625	$86.0935 \pm .1646$
M_Q	78.9938 ± 0.2873	78.8422 ± 0.2857	78.8496 ± 0.2813
σ_I	$34.7688 \pm .7475$	$34.1918 \pm .7351$	$29.2512 \pm .6289$
σ_G	$4.3963 \pm .0945$	$4.1950 \pm .0902$	$4.2732 \pm .0919$
σ_H	$4.6896 \pm .1008$	$4.6920 \pm .1009$	$4.6444 \pm .0999$
σ_R	$5.4323 \pm .1168$	$5.3638 \pm .1153$	$5.4133 \pm .1164$
σ_Q	$9.4466 \pm .2031$	$9.3940 \pm .2020$	$9.2495 \pm .1989$
r_{IG}	$.5989 \pm .0195$	$.6256 \pm .0175$	$.6267 \pm .0185$
r_{IH}	$.4218 \pm .0250$	$.4403 \pm .0245$	$.4388 \pm .0245$
r_{IR}	$.4279 \pm .0249$	$.4433 \pm .0242$	$.4397 \pm .0245$
r_{IQ}	$.4602 \pm .0240$	$.4666 \pm .0240$	$.4689 \pm .0237$
r_{GH}	$.2364 \pm .0285$	$.2647 \pm .0282$	$.2671 \pm .0282$
r_{GR}	$.2210 \pm .0292$	$.2266 \pm .0288$	$.2266 \pm .0288$
r_{HQ}	$.2629 \pm .0282$	$.2745 \pm .0279$	$.2795 \pm .0280$
r_{HR}	$.8270 \pm .0095$	$.8503 \pm .0078$	$.8557 \pm .0081$
r_{HQ}	$.4670 \pm .0240$	$.4836 \pm .0234$	$.4837 \pm .0233$
r_{RQ}	$.4221 \pm .0250$	$.4321 \pm .0248$	$.4356 \pm .0246$
R_{Q-IGH}	$.5503 \pm .0212$	$.5603 \pm .0208$	$.5619 \pm .0208$

TABLE II. The Statistical Constants and Their Probable Errors After Sheppard's Correction

Constant	Coded		Uncoded
	10 classes	20 classes	
σ_I	$34.0656 \pm .7324$	$31.0716 \pm .6680$	$29.2512 \pm .6289$
σ_G	$4.3212 \pm .0929$	$4.1769 \pm .0898$	$4.2732 \pm .0919$
σ_H	$4.6089 \pm .0991$	$4.6745 \pm .1005$	$4.6444 \pm .0999$
σ_R	$5.3715 \pm .1155$	$5.3486 \pm .1150$	$5.4133 \pm .1164$
σ_Q	$9.3078 \pm .2001$	$9.3590 \pm .2012$	$9.2495 \pm .1989$
r_{IG}	$.6219 \pm .0186$	$.6308 \pm .0183$	$.6267 \pm .0185$
r_{IH}	$.4380 \pm .0246$	$.4440 \pm .0244$	$.4388 \pm .0245$
r_{IR}	$.4417 \pm .0245$	$.4467 \pm .0243$	$.4397 \pm .0245$
r_{IQ}	$.4767 \pm .0235$	$.4706 \pm .0237$	$.4689 \pm .0237$
r_{GH}	$.2447 \pm .0286$	$.2669 \pm .0282$	$.2671 \pm .0282$
r_{GR}	$.2274 \pm .0288$	$.2283 \pm .0288$	$.2266 \pm .0288$
r_{GQ}	$.2715 \pm .0282$	$.2767 \pm .0281$	$.2795 \pm .0280$
r_{HR}	$.8510 \pm .0084$	$.8559 \pm .0081$	$.8557 \pm .0081$
r_{HQ}	$.4843 \pm .0233$	$.4872 \pm .0232$	$.4837 \pm .0233$
r_{RQ}	$.4332 \pm .0247$	$.4349 \pm .0247$	$.4356 \pm .0246$
r_{Q-IGHR}	$.5620 \pm .0208$	$.5643 \pm .0207$	$.5619 \pm .0208$

CALCULATION AND USE OF THE STANDARD DEVIATION OF PARTIAL REGRESSION COEFFICIENT

A. E. BRANDT

From the Department of Mathematics, Iowa State College

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Perhaps the most fundamental concept of all statistical work is that of an infinite population from which more or less random samples are drawn. Any set of data or sample represents a limited experience gained from, for example, a fertility plot, a feed lot, or a breeding plot or pen. From this sample we may obtain some idea of the nature of the infinite population from which it is assumed to have been drawn and so of the probable nature of future samples. If a future sample does not agree with the expectations, it is probable that it is drawn from a different population, that is, that there is a material difference in the treatment, in the objects of the treatment, or in the methods of measuring results.

Thus we see there are two distinct problems. One of them is to determine the probability that the various statistical constants of the theoretical population will not differ from the corresponding calculated constants of the sample by more than the ratio of the calculated constant to its standard deviation, and the other is to determine the significance of differences between corresponding constants obtained from two samples. The second problem, in other words, is to determine the probability that the samples have or have not been drawn from the same population. The standard deviation (σ) lends itself to the solution of these problems.

In the illustration which follows we solve only the first problem because we have but one sample. If we assume that a given partial regression coefficient (b -coefficient) is the mean of a normally distributed population and that the distance of this mean from zero is the ratio of the given b -coefficient to its standard deviation (b/σ_B), the probability that the corresponding b -coefficient of the population will not differ from that of the sample by more than the ratio b/σ_B may be read directly from Table II., Tables for Statisticians and Biometricians, edited by Karl Pearson. Thus, in using the table, we first calculate the ratio b/σ_B which is the x of the table; then, entering the table with this value, we read the corresponding value of $\frac{1}{2}(1 + \alpha)$ which is the probability that the corresponding b -coefficient of the population will not differ by more than b/σ_B from the one given. The assumption of normal distribution is valid only for large samples as has been demonstrated by Fisher, R. A. (1922). For small samples Student's tables should be used, Student (1917).

Our present illustration consists of 512 observations on four independent variables (A, B, C, D,) and the dependent variable X. The multiple correlation coefficient ($R_{X,ABCD}$), the regression equation, and the four b -coefficients (b_{XA} , b_{XB} , b_{XC} , b_{XD}), are calculated by the tabular method presented by Wallace, H. A. and Snedecor, George W. (1925). These calculations and results are given in Tables I. and II. In order to determine

the standard deviations of the four b-coefficients by this method, it is necessary also to calculate the four (n-1) order multiple correlation coefficients, that is, the multiple correlation coefficient of each independent variable with the other three after dropping the dependent variable. We represent these coefficients as follows: $R_{D \cdot ABC}$, $R_{C \cdot ABD}$, $R_{B \cdot ACD}$, $R_{A \cdot BCD}$. These calculations and results appear in Tables III., IV., V., and VI. respectively.

With the above results we are prepared to calculate the desired standard deviations. The formula we use is a modification of one presented by Kelley, Truman L. (1923) which we have adapted to the notation and machine methods of Wallace and Snedecor (1925). The formula as originally presented by Kelley (1923) is:

$$\sigma_B(01 \cdot 23 \dots n) = \frac{\sigma(0 \cdot 123 \dots n)}{\sigma(1 \cdot 23 \dots n) \sqrt{N}}$$

which may be read,—the standard deviation of the partial regression coefficient of the dependent variable on the first independent variable is equal to the standard error of estimate of the dependent variable on all of the independent variables divided by the product of the standard error of estimate of the first independent variable on the other independent variables, and the square root of the number of observations. In accordance with a suggestion made by Dr. Kelley in a letter of November 30, 1927, the second factor in the denominator was changed to $\sqrt{N-n}$, n being the number of variables entering into the problem.

In presenting the above formula, Kelley (1923) gave the following relations:

$$\sigma(0 \cdot 123 \dots) = \sigma(0) \sqrt{1 - R^2_{(0 \cdot 123 \dots n)}}$$

$$\sigma(0 \cdot 23 \dots) = \sigma(0) \sqrt{1 - R^2_{(0 \cdot 23 \dots n)}}$$

Using the above relations and the suggested correction, we have:

$$\sigma_B(01 \cdot 23 \dots n) = \frac{\sigma(0) \sqrt{1 - R^2_{(0 \cdot 123 \dots n)}}}{\sigma(0) \sqrt{1 - R^2_{(0 \cdot 23 \dots n)}} \sqrt{N-n}}$$

or, using the notation of Wallace and Snedecor (1925):

$$\sigma_{b_{XA}} = \frac{\sigma_X \sqrt{1 - R^2_{X \cdot ABCD \dots}}}{\sigma_A \sqrt{1 - R^2_{X \cdot ABCD \dots}} \sqrt{N-n}}$$

This form seems to be the one best fitted to our method of solution.

The next step in our illustration is to substitute the appropriate values from Tables I. to VI. inclusive in the above formula and solve for the standard deviation of each of the four b-coefficients. The arithmetic and results of these calculations are given in Table VII.

Now that we have the b-coefficients, given in the regression equation at the end of Table II, and their standard deviations, given in Table III, we are ready to obtain some idea of the nature of the theoretical infinite population in-so-far as the b-coefficients are concerned, which is the first of the two problems. This information is contained in the following table:

Coefficient	Value of coefficient	Standard deviation	$x = \frac{b}{\sigma_B}$	Probability differences will not
				Exceed $\frac{b}{\sigma_B}$ $\frac{1}{2}(1 + a)$
b_{XA}	.0467	.0117	3.99	.9999670
b_{XB}	.0244	.0058	4.21	.9999872
b_{XC}	-.0540	.0088	-6.14	.999999990
b_{XD}	.9848	.0088	111.91	Certainty

The probabilities given in the last column indicate that our sample is representative of the infinite population of which it is theoretically a part. This information gives us confidence that our sample may well be used for comparison with samples similarly drawn to detect whether or not they are drawn from the same population.

By examining the formula we are able to form a basis for judgment so that we will not have to calculate the standard deviations for all samples. Since the $\sqrt{N} - n$ is a factor in the denominator of the formula, it is apparent that the size of the samples has a large influence on the standard deviation of the various b-coefficients. In a large sample in which the standard deviations of the variables are moderate in size, it is reasonable to expect that a b-coefficient of the sample will not differ significantly from that of the population. However, in small samples or in samples in which the dispersion is great, each b-coefficient should be compared with its standard deviation before attaching a great deal of significance to it.

TABLE I. CALCULATION OF CORRELATION COEFFICIENTS AND STANDARD DEVIATIONS.

		A	B	C	D	X
Sums		2560	5120	2048	2048	2048
Means		5	10	4	4	4
A	1	13824	26940	10140	10108	10196
	2	12800	25600	10240	10240	10240
	3	1024	1340	100	132	44
	4	32	2048	1024	1024	1024
	5		.6543	-.0977	-.1289	-.0430
B	1		55296	20470	20288	20454
	2		51200	20480	20480	20480
	3		4096	10	192	26
	4		64	2048	2048	2048
	5			-.0049	-.0938	-.0127
C	1			9216	8173	8113
	2			8192	8192	8192
	3			1024	19	79
	4			32	1024	1024
	5				-.0186	-.0771
D	1				9216	9190
	2				8192	8192
	3				1024	998
	4				32	1024
	5					.9746
X	1					9216
	2					8192
	3					1024
	4					32
S Dev		1.4142	2.8284	1.4142	1.4142	1.4142

TABLE II. SOLUTION OF NORMAL EQUATIONS OF (nth) ORDER ON X

Block	Line	A	B	C	D	X	S
A	1	1.0000	.6543	-.0977	-.1289	-.0430	1.3847
	2	-1.0000	-.6543	.0977	.1289	.0430	
B	3		1.0000	-.0049	-.0938	-.0127	1.5429
	4		-.4381	.0639	.0843	.0281	-.9060
	5		.5719	.0590	-.0095	.0154	.6369
	6		-1.0000	-.1032	.0166	-.0269	-1.1137
C	7			1.0000	-.0186	-.0771	.8017
	8			-.0095	-.0126	-.0042	.1353
	9			-.0061	.0010	-.0016	-.0657
	10			.9884	-.0302	-.0829	.8713
	11			-1.0000	.0307	.0842	-.8851
D	12				1.0000	.9746	1.7333
	13				-.0166	-.0055	.1785
	14				.0002	.0003	.0106
	15				-.0009	-.0025	.0267
	16				.9823	.9669	1.9491
	17				-1.0000	-.9843	-1.9842
Beta(XD) =					.9843	.9843	
Beta(XC) =					-.0540	-.0842	
Beta(XB) =					.0163	.0269	
Beta(XA) =					.1269	-.0430	

$$R^2 = (.0467)(-.0430) + (.0488)(-.0127) + (-.0540)(-.0771) + (.9848)(.9746)$$

$$R = .9805$$

$$\begin{aligned} \bar{X} = 4 + .0467 \frac{1.4141}{1.4142} (A - 5) + .0488 \frac{1.4142}{2.8284} (B - 10) \\ - .0540 \frac{1.4142}{1.4142} (C - 4) + .9848 \frac{1.4142}{1.4142} (D - 4) \end{aligned}$$

$$\bar{X} = .0467 A + .0244 B - .0540 C + .9848 D - 2007$$

TABLE III. SOLUTION OF NORMAL EQUATIONS OF (N-1) ORDER ON D

Block	Line	A	B	C	D
A	1	1.0000	.6543	-.0977	-.1289
	2	-1.0000	-.6543	.0977	.1289
B	3		1.0000	-.0049	-.0938
	4		-.4281	.0639	.0843
	5		.5719	.0590	-.0095
	6		-1.0000	-.1032	.0166
C	7			1.0000	-.0186
	8			-.0095	-.0126
	9			-.0061	.0010
	10			.9844	.0302
	11			-1.0000	.0307
Beta(DC) =				-.0307	-.0307
Beta(DB) =			-.0134	.0032	-.0166
Beta(DA) =		-.1231	.0088	-.0030	-.1289

$$R^2 = (-.1231)(-.1289) + (.0134)(-.0938) + (-.0307)(-.0186) = .0177$$

TABLE IV. SOLUTION OF NORMAL EQUATIONS (n-1) ORDER ON C

Block	Line	A	B	D	C	S
A	1	1.0000	.6543	-.1289	-.0977	1.4277
	2	-1.0000	-.6543	.1289	.0977	
B	3		1.0000	-.0938	-.0049	1.5556
	4		.4281	.0843	.0639	-.9341
	5		.5719	-.0095	.0590	.6215
	6		-1.0000	.0166	-.1032	
D	7			1.0000	-.0186	.7587
	8			-.0166	-.0126	.1840
	9			-.0002	.0010	.0103
	10			-1.0000	.0307	
Beta(CD) =				-.0307	-.0307	
Beta(CB) =			.1027	-.0005	.1032	
Beta(CA) =		-.1689	-.0672	-.0040	-.0977	

$$R^2 = (-.1689)(-.0977) + (.1027)(-.0049) + (-.0307)(-.0186) = .0166$$

TABLE V. SOLUTION OF NORMAL EQUATIONS OF $(n-1)$ ORDER ON B

Block	Line	A	D	C	B	S
A	1	1.0000	— .1289	— .0977	.6543	1.4277
	2	—1.0000	.1289	.0977	— .6543	
D	3		1.0000	— .0186	— .0938	.7587
	4		— .1066	— .0126	.0843	.1840
	5		.9834	— .0312	— .0095	.9427
	6		—1.0000	.0317	.0097	
C	7			1.0000	— .00499	.8788
	8			— .0010	— .0003	.0294
	9			— .0095	.0638	.1395
	10			.9895	.0587	1.0477
	11			—1.0000	— .0593	
Beta(BC) =				.0593	.0593	
Beta(BD) =			— .0078	.0019	— .0097	
Beta(BA) =		.6591	— .0010	.0058	.6543	

$$R^2 = (.6591)(.6543) + (-.0078)(-.0938) + (.0593)(-.0049) = .4317$$

TABLE VI. SOLUTION OF NORMAL EQUATIONS OF $(n-1)$ ORDER ON A

Block	Line	D	C	B	A	S
D	1	1.000	— .0186	— .0938	— .1289	.7587
	2	—1.0000	.0186	.0938	.1289	
C	3		1.0000	— .0049	— .0977	.8788
	4		— .0003	— .0017	— .0024	.0141
	5		.9997	— .0066	— .1001	.8829
	6		—1.00000	.0066	.1001	
B	7			1.0000	.6543	1.5556
	8			— .0088	— .0121	.0712
	9			— .0000	— .0007	.0059
	10			.9912	.6415	1.6327
	11			—1.0000	— .6472	
Beta(AB) =				.6475	.6475	
Beta(AC) =			— .0958	.0043	— .1001	
Beta(AD) =		— .0700	— .0018	.0607	— .1289	

$$R^2 = (-.0700)(-.1289) + (-.0958)(-.0977) + (.6547)(.6543) = .4468$$

TABLE VII. STANDARD DEVIATIONS OF b-COEFFICIENTS AND THEIR CALCULATION

$$\begin{aligned}
 \sigma_{b_{XA}} &= \frac{\sigma_X \sqrt{1 - R^2_{X \cdot ABCD}}}{\sigma_A \sqrt{1 - R^2_{A \cdot BCD}} \sqrt{N - n}} \\
 &= \frac{1.4142 \sqrt{1 - .9614}}{1.4142 \sqrt{1 - .4468} \sqrt{512 - 5}} = .0117 \\
 \sigma_{b_{XB}} &= \frac{\sigma_X \sqrt{1 - R^2_{X \cdot ABCD}}}{\sigma_B \sqrt{1 - R^2_{B \cdot ACD}} \sqrt{N - n}} \\
 &= \frac{1.4142 \sqrt{1 - .9614}}{2.8284 \sqrt{1 - .4317} \sqrt{512 - 5}} = .0058 \\
 \sigma_{b_{XC}} &= \frac{\sigma_X \sqrt{1 - R^2_{X \cdot ABCD}}}{\sigma_C \sqrt{1 - R^2_{C \cdot ABD}} \sqrt{N - n}} \\
 &= \frac{1.4142 \sqrt{1 - .9614}}{1.4142 \sqrt{1 - .0166} \sqrt{512 - 5}} = .0088 \\
 \sigma_{b_{XD}} &= \frac{\sigma_X \sqrt{1 - R^2_{X \cdot ABCD}}}{\sigma_D \sqrt{1 - R^2_{D \cdot ABC}} \sqrt{N - n}} \\
 &= \frac{1.4142 \sqrt{1 - .9614}}{1.4142 \sqrt{1 - .0177} \sqrt{512 - 5}} = .0088
 \end{aligned}$$

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STUDIES ON THE TOXICITY OF HYDROCYANIC ACID¹

JAMES B. ALLISON²

From the Department of Chemistry, Iowa State College.

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I.

The validity of conclusions concerning the toxicity of various substances on organisms depends upon the methods of securing and interpreting the experimental data. Experiments have been made on the toxicity of hydrogen cyanide to a species of weevil, cockroach and the rat and the collected data have been presented in a way which avoids some possible errors in interpreting toxic effects. The work which is reviewed in this paper was completed in the spring of 1927 and is in harmony, in part, with the recent published results of Brinley and Baker (1927). However, these authors report a "supertoxicity" of gas obtained from liquid hydrogen cyanide over the gas generated from calcium cyanide. The data presented here do not support his conclusion.

II.

The apparatus was designed similar to those used in war gas experiments. Fig. 1 is a diagram of the apparatus used to determine the toxicity of hydrocyanic acid gas generated from "Calcyanide." To illustrate the operation of the apparatus the course of the air and gas will be traced. As indicated by the arrow, air enters the generating train at (a). Tube (c) acts as a pressure regulator. It consists of a Carius tube with mercury in the bottom (the darkened portion) and a head of water through which excess air bubbles. Screw clamp (b) aids in regulating the flow of air. In bottles (d) and (e) the air bubbles through sulphuric acid of sufficient density to produce sixty per cent humidity. It is then forced through glass wool in bottle (f) to take up acid spray. Next it flows over sixty grams of "Calcyanide" distributed through tube (g). Here the moist air generates hydrocyanic acid from the easily hydrolyzed calcium cyanide. As the gas air mixture is forced through the flowmeter (h) the rate of flow can be determined and fixed at some mark. For the experiments recorded in this paper, a rate of forty liters per hour was generally used. The concentration of hydrocyanic acid can be determined by taking a sample through tube (i).

It is often necessary to dilute with more air, hence the y tube (k) permits part of the gas to be forced out of the laboratory into the open and part through flowmeter (l). The rate of flow can be regulated by screw clamps (m) and (n). If it is necessary to dilute the gas with more air

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²Now with the Department of Physiology and Biochemistry, Rutgers University.

in order to obtain a desired concentration, screw clamp (o) may be opened, which will connect the dilution train with the system.

The diluted air-hydrocyanic acid-gas mixture now passes into mixing bottle (u). Excess gas mixture is permitted to escape into the open (exit). The amount which is needed for the experiment is drawn by a water suction pump into mixing bottle (w), through flowmeter (x), into exposure bottles (y) and through flowmeter (z). Tube (s) is a suction regulator. The rate of flow can be controlled by screw clamp (t) and can be determined by flowmeters (x) and (z). Any leak in the exposure train can also be detected since the two flowmeters should indicate the same rate of flow. Samples for analysis may be taken from (r).

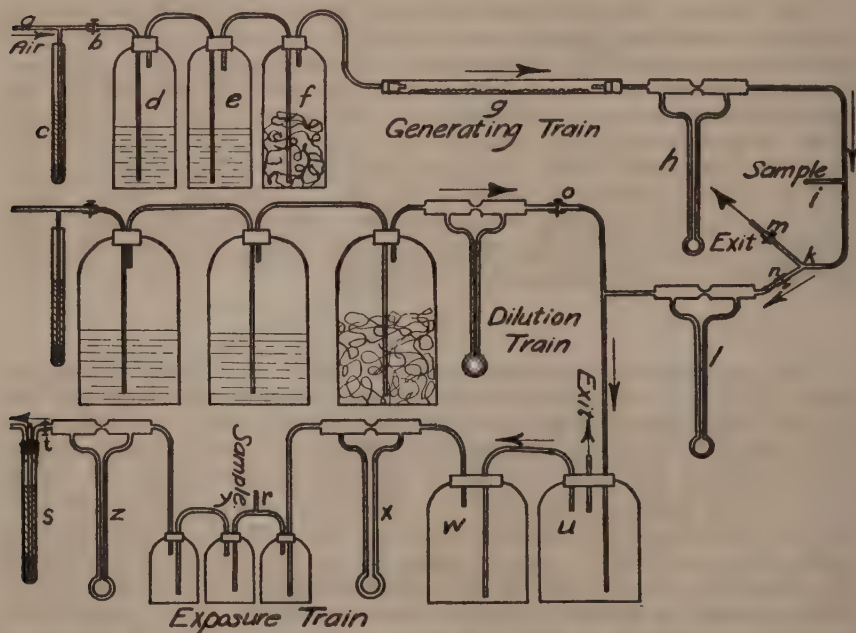


Fig. 1. Apparatus used to determine toxicity of HCN generated from calcyanide.

In all the experiments on toxicity when "Calycyanide" was used, 60 grams were distributed evenly in tube (g). This tube had a three-fourths inch bore and a length of four feet. Air (60% humidity) was passed continually through the tube, usually at the rate of forty liters per hour for three to four days. The first and second day the gas mixture obtained was generally diluted with air until the required concentration of hydrogen cyanide was reached. In most cases no dilutions were made the third and fourth day.

In order to obtain hydrocyanic acid gas from the liquid acid another generating train was substituted in the apparatus described above. Air dried by passing it through calcium chloride and concentrated sulphuric acid was bubbled through liquid hydrocyanic acid kept at a definite temperature. In most of the experiments the liquid was kept at approximately zero degrees centigrade by surrounding the container, which was

inserted in a thermos bottle, by ice. The air saturated with hydrocyanic gas then passed through a flowmeter which can be considered as replacing (1) in the diagram. The rate of flow could then be determined and the gas mixture diluted to the desired strength as before. Since the vapor pressure of liquid hydrocyanic acid is known for various temperatures (Perry and Porter, 1926) it is possible to calculate the amount of gas picked up by the air and to make dilutions accordingly.

Samples of gas were analyzed by the thiocyanate method of Francis and Connel (1913) and by the standard Liebig method. The latter was found to be much simpler and more dependable than the colorimetric. It was found that the use of a solution of silver nitrate (1 cc. = 1 mg. HCN), stronger than that frequently used, and a microburette, makes it possible to obtain a good end point and at the same time to retain the accuracy sought by using a more dilute titrating solution.

The cockroach (*periplaneta americana* L.), the rice weevil (*Sitophilus oryza* L.), and the albino rat, weighing from 55 to 95 grams, were used in this work. Percentage kill was based on counts made at the time when maximum recovery was attained. Only animals which were apparently recovered were counted as living. For example, cockroaches which could move but could not walk after forty-eight hours had elapsed since they had been removed from exposure to the gas, were considered dead. Experience showed that they never fully recovered and later died.

All of the toxicity experiments recorded were carried out at room temperature varying from 23° to 28° C.

III.

There are many methods used to express results of toxicity experiments. There is the mortality rate curve so commonly used in the study of vital statistics and its integral which may be called a time course curve (see Fig. 2 and Fig. 3). Campbell (1926) has devised another method by which he plots, "speed of toxic action," against dosage, "speed of toxic action" meaning one hundred divided by the survival time. Economic entomologists often compare the effect of a toxic gas on different insects and also indicate the relative value of various toxic gases in the following way. A single time of exposure is held constant and the minimum lethal dose is determined. Tattersfield and co-workers, who worked with the effect of different sprays on *Aphis rumiculus* L., developed a method which could be used for gaseous poisons. By holding the time of exposure constant and by varying the concentration used, the per cent kill at each concentration could be determined. The resulting curve would indicate how toxicity varies with concentration. They claim the best point for comparison between different poisons is the concentration which kills 50 per cent.

As previously stated, one of the purposes of this paper is to indicate a method of study of toxicity experiments. The time course curve, like the one in Fig. 2, is very common in biological literature. The sigmoid curve of the physiologist and the logarithmic curve of the bacteriologist are examples. That their shape is affected by many factors is evident. In bacteriological work a change in the reaction of the medium, and in insecticidal feeding experiments the contents of the stomach at time of poisoning, etc., would affect the course of the curve. The factor which

is usually stressed is the variation and distribution of resistances. The shape of the curve is probably due to a summation of all the factors which enter into the reaction of an organism with its "poisoned" environment. Its chief value would seem to be expressed in the last statement.

Such curves would be determinants for those of the type shown in Fig. 4. In this case we have picked the one hundred "per cent kill" point for

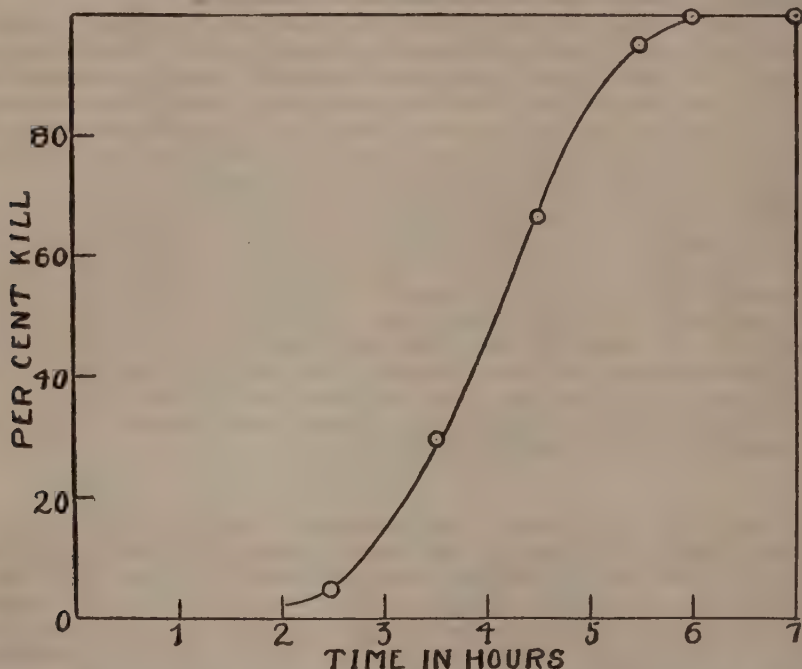


Fig. 2. Time course curve for the weevil (*Sitophilus oryza* L.) exposed to 10,460 parts of HCN to a million parts of gas-air mixture. One hundred selected weevils were used to determine each point. Temperature was 25° C. An approximate equation for this curve is

$$y = \frac{0.1e^{5/3x}}{0.9 + 0.001e^{5/3x}}$$

comparison. Brinley and Baker have pointed out the significance of the latter type of curve for the dosage scale, i. e., there is a limit to the concentration which would be of practical value. The equation, "toxicity = concentration \times the time" will hold within certain limits which depend upon the course of the curve. The range to which it will apply will vary considerably; for instance, the variation shown by curves, a, b, and c. The method of comparing fumigants by determining the concentration necessary to produce one hundred per cent kill at one set time is open to criticism. It may only be accurate for the time of exposure used. For example, a certain concentration of carbon disulphide and the same amount of ethyl formate may give one hundred per cent kill in twenty-four hours,

while at a higher concentration one may be a much more or a less efficient insecticide than the other. Finally, the large differences in resistance to the various gases shown by the various experimental animals should be noted.

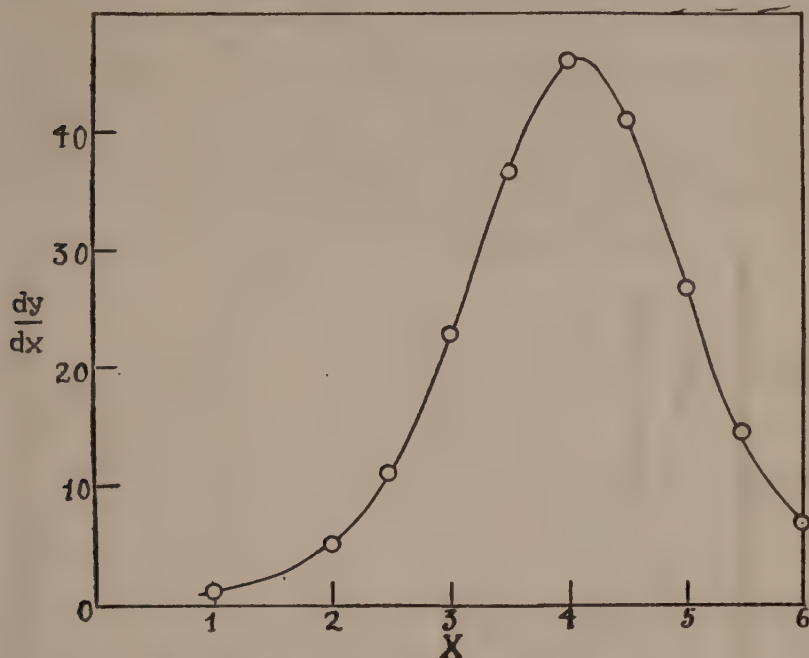


Fig. 3. Mortality rate curve obtained from the equation

$$\frac{dy}{dx} = \frac{5}{3} \frac{0.1e^{5/3x}}{(0.9 + 0.001e^{5/3x})^2}$$

which is the differential of the equation under Fig. 2.

It is believed that by combining the two types of curves used in the work a summary of the action of a poison on an organism can be made. This is done in Fig. 5 from data collected on the toxicity of hydrogen cyanide to the cockroach. The figure is plotted in three dimensions and includes the factors, time, concentration, and per cent kill which are commonly treated. The curve (a c b) is the same as the one shown in Fig. 4. Curve (c n d) is the same type as shown in Fig. 2. A comparison between concentrations and time at any per cent kill can be made. This is done by the use of curve (e n f) which is drawn through the points for sixty per cent kill. It should be noted that the slope of the time course curve such as (c n d) is affected by concentration and the surface which it defines is similarly affected. It is obvious that changes in the course of such curves produced by factors such as variations in resistance would become more apparent at the lower concentrations. The value of the solid

figure can be summarized for the discussion of one series of toxicity tests when experimental conditions are kept constant. A study made on just a section of the figure might lead to erroneous conclusions.

To illustrate the construction of figure 5, a dotted line *gc* is drawn from the *y* axis to the one hundred percent kill curve *a b c*. The distance of this line from the *x* axis indicates a concentration of 69 parts HCN per million. The time necessary to produce one hundred per cent kill at this concentration is found from the intersection of the dotted line *ch* with the axis. From points on the dotted line *gmd* which represent 20, 40, 60 and 80 per cent kill, dotted lines are drawn parallel to the base of the figure to the curve end. Take for example *mn*. It can be seen that a straight edge held parallel to the *y* axis and touching the point of intersection of the dotted lines *mn* and *gmd* will also touch the point on the *z*

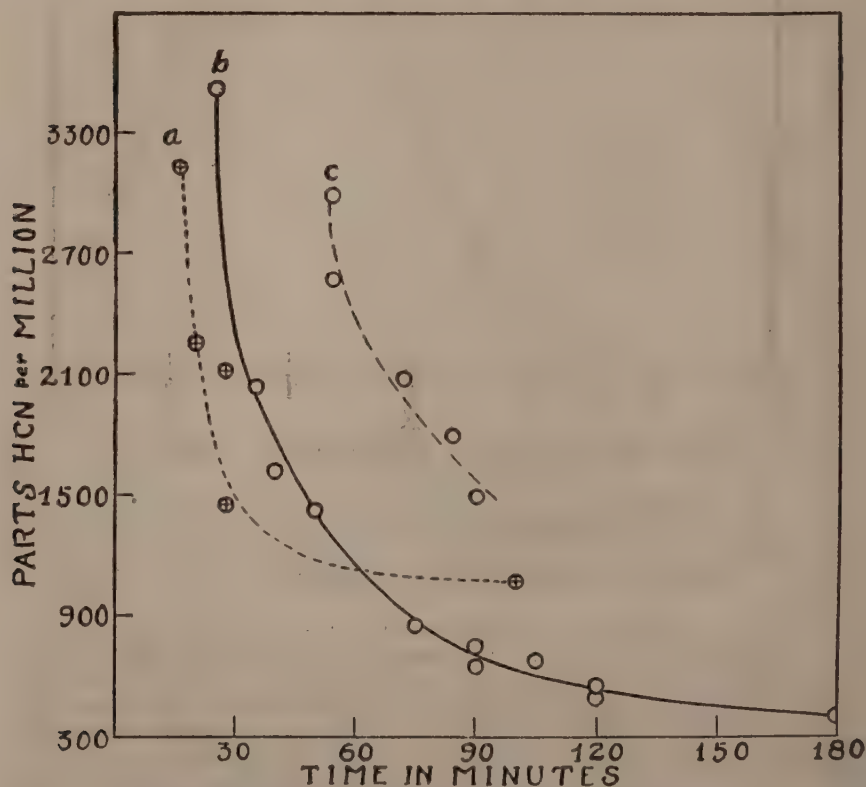


Fig. 4. Concentrations are plotted against the time interval necessary to produce 100 per cent kill. Curve *a* is for the albino rat, weighing from 55 to 95 grams, *b* for the cockroach (*Periplaneta americana* L.) and *c* for the rice weevil (*Sitophilus oryza* L.). The cockroach curve is plotted against the axis as indicated. In order to express the other curves on the same chart, it was necessary to multiply the values obtained for the rat by four and to divide those obtained for the weevil by five.

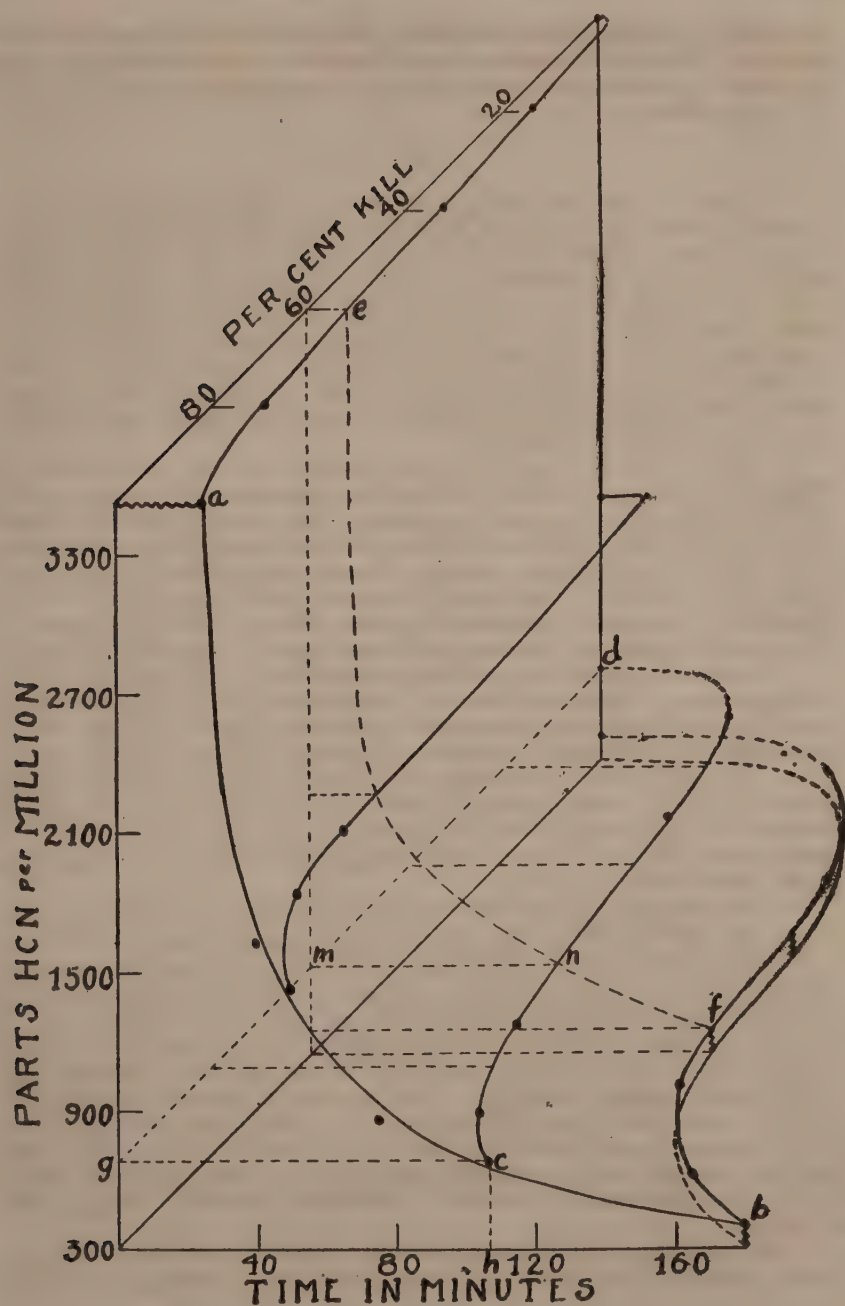


Fig. 5. Effect of HCN on cockroach.

axis which indicates 60 per cent kill. The length of the line $m\ n$ when compared with units of time expressed on the x axis indicates the time necessary to produce 60 per cent kill at a concentration of 689 parts of HCN per million.

IV.

A review of the literature shows that the dosage scale for calcium cyanide in many cases is much less than when either liquid hydrocyanic acid or sodium cyanide is used as a source for the gas. Quayle (1927) discusses some of the probable causes for this apparent "supertoxicity," of the gas obtained from calcium cyanide. During the progress of the work presented here, data were obtained to determine if there was a "supertoxicity" of hydrogen cyanide according to source under carefully controlled experimental conditions and also to discover if the gases obtained from liquid hydrogen cyanide and calcium cyanide were isomeric from the chemical standpoint.

The latter problem suggested itself because of the recent work of Enklaar (1923) who identified two isomeric cyanides by the formation of two isomeric methyl mercuric cyanides. The methyl mercuric iodide needed for the work was prepared from mercuric iodide and methyl iodide by the use of the Grignard reagent. It was recrystallized several times from methyl alcohol. This methyl mercuric iodide (M. P. $45^{\circ}\text{C}.$) was converted to the methyl mercuric hydroxy (M. P. $96^{\circ}\text{C}.$) by means of silver oxide in alcoholic solution, the method used by the above author.

In order to develop the necessary technique it was thought best to duplicate some of Enklaar's results. The reaction of silver cyanide with methyl mercuric iodide in alcohol described in his paper was tried. From the reaction he obtained a low melting methyl mercuric cyanide (M. P. $60^{\circ}\text{C}.$). In our work the resulting methyl mercuric cyanide often melted from 65° to 72° although higher melting derivatives were sometimes obtained. If taken up in ether several times, or allowed to stand on a watch crystal for several hours, the melting point would rise. The melting points varied from 65° to $92^{\circ}\text{C}.$

It was thought possible that the lower melting derivative might be due to the addition of solvent and not to an isomeric change. However, by taking up the higher melting derivative with alcohol and by removing the solvent in a vacuum desiccator, as was done in the preparation, the lower melting derivative was never obtained. After the melting point of the lower melting derivative was determined, the mixture was allowed to recrystallize in the capillary tube and the melting point retaken. The melting point changed very little. The question of impurities was investigated but none were found. If the reaction is carried out in benzene near the freezing point of the solvent, when the solvent is removed the methyl mercuric cyanide would sometimes come out as a colorless syrup which would crystallize quickly if stirred. Indications pointed to the presence of isomers.

The technique used to study the hydrocyanic acid gasses was the same as described by Enklaar. By passing the gas generated from liquid

hydrocyanic acid through the apparatus as for a toxicity experiment, then into an alcoholic solution of methyl mercuric hydroxide, the higher melting methyl mercuric cyanide predominated, (M. P. 89°-91°C.). The gas generated from "Calcyanide" was treated in a similar manner and again the higher melting derivative was obtained (M. P. 91°-92°C.). It should be noted that the derivative obtained from "Calcyanide" generally melted slightly higher than the other but the difference was not sufficient to warrant a conclusion that any apparent "supertoxicity" of one gas over the other was due to isomeric hydrocyanic acids.

The killing properties of the two gases were next investigated. It was soon found that the points for one hundred per cent kill resulting from the use of either source of the gas would fit into a smooth curve. See the data in Table I. The cockroach (*Periplaneta americana* L.) was used to obtain this data and it is included in curve a, Fig. 4. Any irregularities which were noted were not consistent enough to point to a "supertoxicity" of either gas and were probably due to biological or other factors.

TABLE I. The Toxicity of Gas from Liquid HCN and from Calcyanide on the Cockroach (*Periplaneta americana* L.).

Conc. of hydrocyanic acid in parts per million	Time in min. necessary for 100% kill	Gas generated from
3523	25	"Calcyanide"
1435	50	"
869	75	Liquid HCN
764	90	"Calcyanide"
689	105	"
660	90	"
563	120	Liquid HCN
505	120	"Calcyanide"
407	180	Liquid HCN

SUMMARY

1. Variations in the shape of the time course curve are probably due to a summation of all the factors which enter into the reaction of living tissue with its "poisoned" environment.

2. Change in concentration of a poison is related to the time necessary to produce a constant per cent kill by curves which are determined by the time course curves. The former are of value for determining the effect of different poisons on one organism or the effect of one poison on different organisms.

3. By combining these two types of curves into a solid figure a summation can be made of numerous series of toxicity data.

4. The data presented in this paper do not indicate a "supertoxicity" or in fact any difference, either chemical or in toxic effect, between gas

generated from liquid hydrocyanic acid and that generated from "Calcyanide."

I am indebted to Dr. R. M. Hixon and Dr. C. J. Drake for helpful advice and encouragement throughout the research upon the toxicity of hydrocyanic acid gas.

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ON CERTAIN POWER SERIES WITH POSITIVE COEFFICIENTS

EDWARD S. ALLEN

From the Department of Mathematics.

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If we attempt to find a power series which will formally satisfy an ordinary differential equation of degree higher than the first, it usually happens that we must obtain each coefficient from a formula involving all the preceding ones. The question of the interval of convergence of power-series defined in such a way has not, I believe, been considered to any extent. The present paper will show some of the methods available for examining particular cases. Whether or not these methods should be of use in establishing a general theory, they will undoubtedly be suggestive of ways of attacking other problems of this type.

I.

If, in the equation

$$Ax^2y'^2 + 2(A + B)xyy' + (A + B + C)y^2 - y' = 0, \quad (1)$$

where A, B, C are constants, we assume that

$$y = a_0 + a_1x + a_2x^2 + \dots + a_nx^n + \dots, \quad (2)$$

we find that the coefficients must satisfy the equation

$$a_n = \sum_{k=0}^{n-1} b_{n,k} a_k a_{n-k-1}, \quad (3)$$

where

$$b_{n,k} = \frac{A(k+1)(n-k) + Bn + C}{n}. \quad (4)$$

It will be noticed that $nb_{n,k}$ is the most general quadratic polynomial in k , in which k and $(n - k - 1)$ are interchangeable.

We will now assume that A, B , and C are non-negative, real constants.

In certain special cases, which will be considered first, the general coefficient can be found in closed form; hence the interval of convergence, or a safe approximation of it, can be found directly.

Case 1. $A = 0$.

Without essential loss of generality, we can assume that $a_0 = 1$. This we shall do throughout this section.

In the case now under consideration

$$a_n = \frac{1}{n!} (B + C) (2nB + 2C) ((2n - 1)B + 3C) \dots \dots \dots ((n + 2)B + nC) \quad (5)$$

Formula (5), easily verified for $n = 1$ or 2 , is proved in general by mathematical induction. If it is assumed correct for subscripts less than n , we derive from (3) and (4)

$$\begin{aligned}
 a_n = \frac{B+C}{n} [nB+C] & \left\{ \frac{1}{(n-1)!} [(2n-2)B+2C][(2n-3)B+3C] \right. \\
 & \dots [(n+1)B+(n-1)C] + (B+C) \frac{1}{(n-2)!} [(2n-4)B \\
 & + 2C][(2n-5)B+3C] \dots [nB+(n-2)C] + \frac{1}{2!} (B+C) \\
 & (4B+2C) \frac{1}{(n-3)!} [(2n-6)B+2C] \dots [(n-1)B+(n-3)C] \\
 & + \dots \dots \dots \\
 & + \frac{1}{(k-1)!} (B+C)[(k+1)B+(k-1)C][(k+2)B \\
 & + (k-2)C] \dots [(2k-2)B+2C] \frac{1}{(n-k)!} [(2n-2k)B+C] \\
 & \left. [(2n-2k-1)B+3C] \dots [(n-k+2)B+(n-k)C] \right\}. \quad (6)
 \end{aligned}$$

The second members of (5) and (6) must now be proved identical. They are both homogeneous polynomials of degree n in B and C . That they differ, at most, by a factor independent of B and C , we prove by showing that, if any factor of the second member of (5) is 0, the same is true of the second member of (6).

The hypothesis will hold true if

$$(n+1+j)B + (n+1-j)C = 0 \quad [j = 0, 1, 2, \dots, n-1]. \quad (7)$$

Let us, then, set $C = -\frac{n+1+j}{n+1-j} B$ in the second member of (6). The result consists essentially of the sum

$$\begin{aligned}
 & \sum_{k=1}^n \frac{1}{(k-1)!} [kj-(n+1)][kj-2(n+1)] \dots [kj-(k-2)(n+1)] \cdot \\
 & \frac{1}{(n-k)!} [(n-k+1)j-(n-k-1)(n+1)][(n-k+1)j \\
 & -(n-k-2)(n+1)] \dots [(n-k+1)j-(n+1)] \\
 & = \sum_{k=1}^n \frac{(-1)^k}{(k-1)!(n-k)!} [(n-k+1)j-(n-k-1)(n+1)]
 \end{aligned}$$

$$\begin{aligned} & [(n-k+1)j - (n-k-2)(n+1)] \quad . \quad . \quad . \quad . \\ & [(n-k+1)j - j(n+1)] [(n-k+1)j - (j-1)(n+1)]^2 \quad . \quad . \quad . \\ & [(n-k+1)j - (n+1)]^2 [(n-k+1)j] \quad . \quad . \quad . \quad . \\ & [(n-k+1)j + (k-j-2)(n+1)] \quad . \end{aligned} \quad (8)$$

If, now, we name

$$n_j - (n - 2) (n + 1) = N_1,$$

and

$$N_1[N_1 + (n+1)] [N_1 + 2(n+1)] \dots$$

our sum can be written

$$\frac{1}{(n-1)!} \sum_{k=1}^n \binom{n-1}{k-1} (-1)^k F_1(N_1 + (k-1)(n-j+1)) \cdot$$

$$[-jk + (n+1)] [-jk + 2(n+1)] \dots$$

$$\dots [-jk + (j-1)(n+1)]. \quad (9)$$

$\binom{r}{s}$ denotes the binomial coefficient $\frac{r(r-1) \dots (r-s+1)}{s!}$.

F_j is a polynomial of degree $(n-j-2)$.

It is important to notice that the product of the brackets in (9) is a linear combination of the following $\binom{j+1}{2}$ numbers, with coefficients independent of k :

$$\begin{aligned}
& 1, \\
& (k-1), \quad (k-1)(k-2), \dots, (k-1)(k-2) \dots (k-j-1), \\
& (n-k), \quad (n-k)(k-1), \dots, (n-k)(k-1) \dots (k-j+2), \\
& (n-k)(n-k-1), (n-k)(n-k-1)(k-1), \dots \\
& \qquad \qquad \qquad (n-k)(n-k-1) \dots (k-j+3), \\
& \dots\dots\dots \\
& (n-k)(n-k-1) \dots (n-k-j+2) \qquad \qquad \qquad (10)
\end{aligned}$$

The proof of this statement involves the fact that $\binom{j}{2}$ is also the number of different products of type kPn^q occurring in the product of brackets in (9). It involves, further, the fact that the determinants of several systems of linear equations, needed in finding the coefficients, are all different from 0. A typical determinant is

$$\begin{vmatrix}
 1 & \sum_1^{j-1} a & \sum_1^{j-1} a_1 a_2 & \dots & \prod_1^{j-1} a \\
 1 & \sum_0^{j-2} a & \sum_0^{j-2} a_1 a_2 & \dots & \prod_0^{j-2} a \\
 \dots & \dots & \dots & \dots & \dots \\
 1 & \sum_{2-j}^0 a & \sum_{2-j}^0 a_1 a_2 & \dots & \prod_{2-j}^0 a
 \end{vmatrix},$$

which equals

$$(-1)^{\binom{j}{2}} 2^2 \cdot 3^3 \dots (j-2)^{j-2} (j-1)^{j-1}.$$

If, now, $\binom{n-1}{k-1}$ is multiplied by any of the numbers (10), the result is the product of a binomial coefficient of similar type, and of factors independent of k .

E. g., $\binom{n-1}{k-1} (n-k)(n-k-1)(k-1)$

$$\begin{aligned}
 &= \frac{(n-1)!}{(k-1)!(n-k)!} (n-k)(n-k-1)(k-1) \\
 &= \frac{(n-1)!}{(k-2)!(n-k-2)!} = \binom{n-1}{k-2} (n-1)(n-2)(n-3).
 \end{aligned}$$

The upper index of the binomial coefficient is reduced by the number of factors involving k ,—at most $(j-1)$; accordingly it remains $(n-j)$ or more.

The terms of (9) being separated into $\binom{j+1}{2}$ sums, according to the linear factors involving k , it turns out that each of these sums is an $(n-j)$ th, or higher, successive difference of a polynomial of degree $(n-j-2)$. The value of (9) is accordingly 0, as the proof required.

The second members of (5) and (6) differ at most, then, by a factor independent of B and C . That this factor is 1 can be seen most easily from the fact that 1 is the coefficient of C^n in both expressions.

Information on the interval of convergence of the series considered can now be obtained from the test-ratio. (5) gives us:

$$\begin{aligned}
 \frac{a_{n+1}}{a_n} &= \left[\frac{n+3}{n+1} B + C \right] \left\{ \left[1 + \frac{2B}{2nB+2C} \right] \left[1 + \frac{2B}{2nB+3C} \right] \right. \\
 &\quad \left. \dots \left[1 + \frac{2B}{2nB+nC} \right] \right\} \quad (11)
 \end{aligned}$$

The limit of the first factor, as n becomes infinite, is $(B+C)$.

The other factors are certainly less than $\left[1 + \frac{2B}{2nB+2C} \right]^{n-1}$, of which the limit is e .

Accordingly the original series converges, at least, for $-e^{-1}(B+C)^{-1} < x < e^{-1}(B+C)^{-1}$.

(12)

This interval of convergence can, of course, be widened by closer attention to $\frac{a_{n+1}}{a_n}$. If, for instance, we consider the effect of replacing the product of two factors equally distant from the ends of the brace in (11) by

$$\left\{ 1 + \frac{2B}{2nB + \left(\frac{n}{2} + 1\right)C} \right\}^2, \text{ we find an interval of convergence extending}$$

as far as

$$\frac{-B(32B^2 + 33C^2)}{(B + C)^{-1} e^{8/(4B+C)} (B^2 + C^2)}$$

Case 2. $B = C = 0$.

$$a_n = \frac{(2A)^n (n+1)^{n-2}}{n!}, \quad (13)$$

as can be established by mathematical induction.

$$\frac{a_{n+1}}{a_n} = \frac{2A(n+2)^{n-1}}{(n+1)^{n-1}}.$$

$$\text{As } \lim_{n \rightarrow \infty} \left(\frac{n+2}{n+1} \right)^{n-1} = \lim_{n \rightarrow \infty} \left[\left(1 + \frac{1}{n+1} \right)^{n+1} \frac{(n+1)^2}{(n+2)^2} \right] = e,$$

the interval of convergence extends to $\pm \frac{1}{2Ae}$.

The series converges at both ends of this interval, since $\left(1 + \frac{1}{n+1} \right)^{n+1} < e$, and $\frac{(n-1)^2}{(n-2)^2}$ is the test-ratio of a convergent series.

We have, then, as the exact interval,

$$-\frac{1}{2Ae} \leq x \leq \frac{1}{2Ae}. \quad (14)$$

Case 3. $C = 0$.

By methods similar to those used in Case 1, the value of the general coefficient

$$a_n = \frac{2^n(n+1)^{n-2}}{n!} (A+B)^{\frac{n+1}{2}} \left[A+B - \left(\frac{n-2}{n+1} \right)^2 B \right]^{\frac{1}{2}} \\ \left[A+B - \left(\frac{n-4}{n+1} \right)^2 B \right]^{\frac{1}{2}} \dots \left[A+B - \left(\frac{-n+2}{n+1} \right)^2 B \right]^{\frac{1}{2}} \quad (15)$$

can be shown to depend on the formula

$$\sum_{k=0}^n \left[\frac{1}{[(n-2p+1)(k+1) + (n+2)k]} \cdot \frac{1}{[(n-2p+1)(n-k+1) + (n+2)(n-k)]} \binom{n}{k} \right. \\ \left. \frac{[(n-2p+1)(k+1) + (n+2)k] [(n-2p+1)(k+1) + (n+2)(k-2)] \dots}{[(n-2p+1)(k+1) - (n+2)(k-2)] [(n-2p+1)(n-k+1) + (n+2)(n-k-2)] \dots} \right] \\ = (-1)^{p+1} \frac{(n+2)^{n-1}}{2^{n-3}(n-2p+1)} \frac{(2n-2p)!(2p-3)!}{(n-p)!(p-2)!} \quad (16)$$

Now this formula is a special case of the much more general one, recently proved by Hasse and Bessel-Hagen:—

$$\sum_{j+k=n} \left[\frac{x}{zj+x} \binom{zj+x}{j} \frac{y}{zk+y} \binom{zk+y}{k} \right] = \frac{x+y}{zn+x+y} \binom{zn+x+y}{n}$$

Their ingenious proof of this identity, which Professor Hasse was so kind as to communicate to me, will appear in the *Jahresbericht der Deutschen Mathematiker-Vereinigung*.

In this formula n, j, k are non-negative integers, such that $j+k=n$, and x, y, z are variables. For our case, we let

$$x=y=\frac{n-2p+1}{2n+4}, \quad z=\frac{2n-2p+3}{2n+4}.$$

Using (15), let us investigate the condition for the convergence of the series. It is convenient to consider the product of two successive test-ratios

$$\frac{a_{n+2}}{a_n} = \frac{\left[A+B-\left(\frac{n}{n+3}\right)^2 B \right] \left[A+B-\left(\frac{n-2}{n+3}\right)^2 B \right] \dots}{\left[A+B-\left(\frac{n-2}{n+1}\right) B \right] \left[A+B-\left(\frac{n-4}{n+1}\right) B \right] \dots} \cdot \frac{(n+3)^n}{4(A+B)(n+1)^{n-1}(n+2)}.$$

The second fractional factor approaches e as n becomes infinite. For an estimate of the first fraction, we consider the series, in powers of $\frac{B}{A+B}$, giving the logarithms of the factors in numerator and denominator. Addition of these series gives a result which is increased when replaced by the approximation for the first two terms,

$$\left(-\frac{1}{3} \frac{B}{A+B} - \frac{n}{10(n+1)} \left(\frac{B}{A+B} \right)^2 \right).$$

Allowing n to become infinite, and using the corresponding exponential

function, we find that $\frac{a_{n+2}}{a_n} < 4(A+B)e^{\frac{6A+5B}{3A+3B}}$, and hence that the power-

series converges when $|x| < \frac{1}{2\sqrt{A+B} e^{\frac{1}{3} - \frac{B}{6A+6B}}}$. (17)

Case 4:

Without finding any closed form for a_n in the case where A , B and C are arbitrary positive numbers, we can readily see that the series is still convergent in the interval (17).

By the addition of C , $b_{n,k}$ is increased in the ratio

$$1 + \frac{C}{A(k+1)(n-k)+nB} \leq 1 + \frac{C}{n(A+B)}.$$

Accordingly, if \bar{a}_1 is obtained from a_1 by the substitution of 0 for C ,

$$\frac{\bar{a}_1}{a_1} < 1 + \frac{C}{A+B} = \lambda_1$$

$$\frac{\bar{a}_2}{a_2} < \left(1 + \frac{C}{A+B}\right) \left(1 + \frac{C}{2(A+B)}\right) = \lambda_2$$

$$\frac{\bar{a}_i}{a_i} < \left(1 + \frac{C}{A+B}\right) \left(1 + \frac{C}{2(A+B)}\right) \dots \left(1 + \frac{C}{i(A+B)}\right) = \lambda_i$$

That is, the terms of

$$a_0 + a_1 x + a_2 x^2 + \dots + a_i x^i + \dots$$

will be no greater than the corresponding ones of

$$\bar{a}_0 + \bar{a}_1 \lambda_1 x + \bar{a}_2 \lambda_2 x^2 + \dots + \bar{a}_i \lambda_i x^i + \dots$$

The test-ratio of the latter series is

$$\frac{\bar{a}_{n+1}}{\bar{a}_n} = x \left(1 + \frac{C}{(n+1)(A+B)}\right);$$

from which we can deduce its convergence for all values of x within the interval (17).

II.

Another power-series, each of whose coefficients depends on all the preceding ones, is obtained as a particular solution of the differential equation

$$2x\left(x\frac{dy}{dx} - y + 1\right)^2 - \frac{dy}{dx} = 0 \quad (18)$$

If we choose that, in the series

$$y = a_0 + a_1x + a_2x^2 + \dots + a_1x^1 + \dots,$$

$a_0 = 0$, it follows that $a_i = 0$ whenever i is odd. The other coefficients are determined by the equation

$$a_{2n} = \frac{1}{n} \left[(2n-3)a_{2n-2} + (2n-5)a_2a_{2n-4} + 3(2n-7)a_4a_{2n-6} + \dots \right. \\ \left. + (2k-1)(2n-2k-3)a_{2k}a_{2n-2k-2} + \dots \right], \quad (19)$$

where $a_2 = 1$.

The interval of convergence is found by comparison with the series

$$1 + \frac{2-s}{2} + \frac{2-s}{2} \cdot \frac{3-s}{3} + \frac{2-s}{2} \cdot \frac{3-s}{3} \cdot \frac{4-s}{4} + \dots,$$

which serves as the basis for Raabe's test, being convergent when $s > 1$.

The series under discussion will converge if

$$a_{2n}x^{2n} < a_4x^4 \cdot \frac{2-s}{2} \cdot \frac{3-s}{3} \cdot \dots \cdot \frac{n-1-s}{n-1}.$$

Assuming this inequality to hold for $n < N$, and setting $\frac{1}{x^2} = \lambda$, we ask under what conditions it will be true also for $n = N$. We find

$$\frac{(N-1)(2N-3)}{(N-1-s)N} \lambda^2 + \frac{(N-2)(N-1)(2N-5)}{(N-2-s)(N-1-s)N} \lambda \\ + \sum_{k=4}^{N-1} \frac{(N-k)(N-k+1) \dots (N-1)}{(N-k-s)(N-k-s+1) \dots (N-1-s)} \cdot \\ \frac{(2-s)(3-s) \dots (k-2-s)}{2 \cdot 3 \cdot \dots \cdot (k-2)} \cdot \frac{(2N-2k-1)(2k-3)}{N} < \lambda^s \quad (20)$$

If, now, we allow N to become infinite, we must make sure that the terms in the summation of the first member approach those of a convergent

series. Comparison with the test-ratio of $\sum \frac{1}{n^u}$ (where $u < 1$) shows that a

sufficient condition for convergence is that $s > \frac{3}{2}$.

The truth of the inequality obtained from (20) by allowing N to become infinite would not, of course, prove (20) itself. It is readily found, however, that the effect of an increase of N by a unit is, in the first place, to increase the number of terms in the first member of (20); and, in the second place, to increase the $(k-1)$ th term by a positive multiple of

$$k^2(2sN-2s) + k(3sN-3N-s) + (2N^3+2N^2-sN).$$

The discriminant of this polynomial in k is negative for $N > 2$; the polynomial, being positive when k is sufficiently large, is always positive. From the truth of the limiting form of (20) we can, then, infer that of (20) itself.

The infinite series in the inequality in question can again be replaced

by an excessive approximation of $\sum \frac{1}{n^u}$ (we may choose $u = \frac{9}{8}$) obtained by

evaluation of $\int_2^\infty \frac{dx}{x^u}$. The resulting inequality is, if s is taken as $\frac{7}{4}$,

$$\lambda^3 - 2\lambda^2 - 2\lambda - 152 > 0.$$

Solution of the corresponding equation shows that this is true, and the original series accordingly convergent, if $|x| < .4$.

THE FORECASTING OF ECONOMIC PHENOMENA

JOHN A. HOPKINS, JR.

From the Department of Agricultural Economics, Iowa State College.

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During the past ten years there has developed a widespread interest in the forecasting of prices and other economic phenomena. Many different methods, some scientific, some semi-scientific, and many entirely empirical, have been used in these efforts to anticipate the future. The results have been as diverse as the methods used. Many have proved themselves inadequately related to the basic forces. A good many have given favorable results under limited sets of conditions, but not a few seem fairly reliable under the varying conditions of the economic world.

It is not surprising that economists and others have shown an active interest in the problem of forecasting. In economic life there are relatively few things that are subject even to a moderate degree of control by the producer or the consumer in his individual capacity. Further, relatively few of the forces beyond his individual control have proven amenable to organized effort. Even here the extension of control is slow and difficult because of the tenacity with which many producers cling to the prerogatives of individual economic freedom.

To the individual, therefore, the immediately practical problem becomes largely one of adjusting himself to the conditions around him. If oats are cheap there is little that he can do toward obtaining a higher price, but he may be able to restrict his acreage of oats and to increase that of some other and more profitable crop. If the price of corn is high and hogs low he can raise fewer hogs and feed them to lighter weights. But there is no way, by positive action, in which he alone can lower the one and raise the other. In fact, while this might be in the interest of one group of producers it would be contrary to the interests of others.

The production of our common economic wares requires a considerable period of time. Hence the adjustment may well come too late and may meet with a maladjustment in the opposite direction. What the producer needs, therefore, is a cue to the reshaping of his plans in time to make them effective while the indicated conditions continue. This implies the use of foresight as to the consequences of activity still in its developmental stages. In short, it implies forecasting.

The layman may well demand that the economist give him some insight into the probable course of events. It is the economist's business to study and attempt to understand economic phenomena. And what general object has the understanding of these forces except to make use of them? If the economist really does comprehend the forces and phenomena with which he is dealing, should he not also perceive their general drift for a short time in the future? It is in the effort to answer this challenge that the methods of forecasting have been or are being developed.

Nature of the Forecasting Problem:

Economic forecasting attempts to anticipate the changes in prices or rates of activity in industry or in other series with economic significance. If we examine some of these series, for instance, the prices of hogs, the production of pig iron, the loading of freight cars, or the volume of bank clearings, we find that each one of them shows a wide, and often abrupt, variation from month to month and from year to year. When plotted on cross section paper such a series of monthly or yearly data shows the irregular sawtooth contour which has become familiar through graphs published in papers and magazines. In these graphs the curves frequently seem to rise and fall erratically from month to month and it may seem hard to discover any really systematic variation.

In many series, however, a more careful examination will show a more or less regular oscillation from year to year, and often in addition a consistent trend upward or downward over a considerable period of time. The continued upward or downward movement is known as the secular trend, while the recurring rise and fall within years is called the seasonal variation. After these more or less regular variations are removed from the data there will be found to remain a series of irregular fluctuations which may or may not contain a rhythmic or cyclical oscillation more or less uniform in periodicity. If such a periodicity is not present the residual fluctuations may appear to have no regular or systematic variations at all. These seemingly erratic variations may account for a large part of the total fluctuation of the series. The question may well be raised as to how it would be possible to forecast with any degree of nearness to the actual facts as they develop from month to month.

Generally a study of related phenomena will suggest the causes for the more outstanding fluctuations in prices or other economic series. Thus variations in crop yields may explain some of the fluctuations in livestock prices at a later time. Variations in the weather during the growing season explain variations in yields and in prices of crops at later periods. A characteristic of economic phenomena is that they are to a very large degree interrelated. Thus in explaining the price of hogs we find one influence to come from the size of the corn crop. But the size of the corn crop affects also the price of cattle, and the price of various other things into which corn enters as a raw material. It also influences the prices of those things for which corn may under some conditions be substituted. There is a close sympathy between the prices of corn and of oats and barley, a more remote one between corn and wheat, and between corn and the forage crops.

In such ways as these the lines of causation in economic activity branch out into a network which unites the entire economic system into an organic whole. Each industry must be regarded as functioning with regard to other industries. Any noteworthy event in one may be expected to have more or less definite repercussions in related fields. Thus a coal strike may be expected to tie up manufacturing and to reduce the traffic on railroads. An invention which cheapens steel may be expected to result in the use of more steel in practically all those industries where this metal serves as a raw material. A rise in the price of beef may be expected to lead to a larger demand for pork and to affect the price of that meat accordingly. The economic world is essentially a unit and this fact goes a long way toward making forecasts of economic development possible.

Economic changes, however, are not to be regarded simply as steps in the reestablishment of a static equilibrium. It is true that if conditions in each industry were to remain unchanged for a considerable period of time we might very well expect a condition in which there would be no further acceleration or retardation of activity. The difficulty with viewing the economic complex in this manner is that no condition approaching the static is ever reached or, because of the nature of the forces and influences concerned, is ever to be expected.

The concept of the supply of a commodity which is easiest to grasp is that of a certain quantity available at one price and of larger quantities available at each higher price. But a moment's reflection will show that this is only part of the truth. The supply might better be regarded as a *rate of flow* than as an amount in existence. If there develops a larger demand for radio sets it will be possible to deliver to consumers only the number already manufactured and existing in the stocks and warehouses at the present time. For a continuously larger delivery it would be necessary to go further back toward the source and increase the rate of flow of the radio sets from the factories. In a similar way if an unusually large output of pork depresses the price, no lasting relief is to be expected to the farmers unless measures proposed reduce the rate of flow of pork from the farms.

On the other side, the simple concept of demand is that of a list of different amounts which will be bought and consumed at different prices, each lower price resulting in the purchase of a larger amount. This is not so far afield as the correspondingly naive concept of supply. It is true that the rate can adjust itself more quickly than supply as far as those consumers are concerned who are already acquainted with the commodity. But the same lowering of price which increases sales to old users is likely to result in some who were previously unacquainted with it gradually adding to the list their demands as they discover the satisfactions to be obtained.

The adjustment is not simply the immediate seeking of a new but previously ascertainable equilibrium. Rather, there is a growth into a new situation in which this simpler adjustment is only one element. The same is true of reactions on the side of the supply to an even greater degree and in a more permanent sense. The increase of a price is likely to result in additions to factories, the building of new stores, the plowing up of new land, or the production of new equipment. These additions to the productive plant, once they are made, may be expected to continue to produce the commodities for which they were intended even after the price, perchance, declines again to the old level or below it. In other words, the initial change in price has more or less permanent influences which themselves compose part of the economic situations in future times.

Here there appears a deeply significant characteristic which differentiates economic phenomena from the mechanical. The economic processes are irreversible. Economic changes propagate themselves forward into new situations which contain elements produced by those changes themselves, and which therefore prevent a turning back to the former situation and prevent identical reactions recurring should the same stimulus be applied again. The element of time which serves as one dimension in economic activity is closely associated with this characteristic.

The opportunity for forecasting comes from the fact that economic reactions contain a large element of inertia and habit. Having continually reacted in an essentially similar manner to similar stimuli, persons continue from inertia to follow paths of action which vary but little in direction or rate of progress within reasonably short periods. Modifications in the economic organism such as those suggested above do not as a usual thing change the nature of the reaction to a given stimulus, but only accelerate or retard them for a limited period of time. Forecasting is permitted by the fact that the reactions may be expected to follow a fairly definite path and to continue for an appreciable period of time.

The Time Factor in Economic Life:

The factor of time is one of the most important in economic activity, and is at the same time one of the most neglected as concerns any explicit statement of its consequences in economic literature. There has been a tendency to regard economic situations in a purely static or cross sectional manner. That is, the efforts to explain price situations have proceeded in the same manner as might be used in explaining the positions of a series of levers and weights in a mechanical contrivance in which a movement of one would be accompanied simultaneously by corresponding changes in others.

But this is far from the case in the economic world. The analogy would be more nearly correct if a time device were attached to the machine so that the moving of a lever would be followed after a time interval by the lifting of a weight, which at some still later time would result in the compression of a piston, and so on at various intervals until the original energy was dissipated.

But analogies are always more or less incomplete and unsatisfactory. It is likely that a better understanding may be gotten from some of the phenomena themselves. Each stimulus applied in economic activity may be expected to have little effect at once, but to set in motion a chain of influences which lead to subsequent events.

Thus if the price of corn is high in the winter and spring it may be expected shortly to curtail the feeding of cattle and hogs and to some extent to reduce the breeding of sows for fall pigs. It would also be expected to result in a greater amount of substitution of oats and other feeding stuffs in place of corn. Also, the high price would naturally be expected to lead to the planting of a somewhat greater acreage of corn. But this would have no direct influence on the supply or price until some six or seven months later. In the meantime, the changing appearance of the growing crop would result in the price rising or falling from week to week.

As time passes still further and the new and larger crop becomes available (assuming favorable weather conditions) the price of corn may be expected to decline as the crop comes to market or its existence becomes evident. Now with lower priced corn there will be a tendency to substitute it in place of other grains. There would be a greater number of cattle put on feed with correspondingly lower cattle prices six or seven months later. We would also expect the breeding of a greater number of sows for the following spring pig crop. This last would result in a larger amount of pork on the market after still another year, that is, eighteen months after the planting of the large corn crop. And finally if the planting of

corn had been overdone the first year and price unduly depressed we might expect some readjustment and restriction in the planting in the second year.

Plainly, the corn, hog and cattle price situations just referred to cannot be considered without regard to the time factor and the length of time that has passed since the stimulus occurred and the readjustment began. In the fall, as the large corn crop begins to be harvested, the situation is not adequately described by pointing out the then existing prices, the amount of corn available for sale, and the numbers of hogs and cattle in the country.

These facts are but passing events resulting from a continuous flow of forces. In a short time each fact will be more or less changed. The corn supply will be increased as the harvest proceeds. The number of hogs will change as the earlier influences on breeding have effect, and will continue to change for some months as the number of pigs farrowed reflects the variations in the corn supply and price which occurred earlier. While the adjustment is in progress and even while its results are being felt, other stimuli, perhaps of a different nature, are pretty sure to occur and set in motion still other processes of adjustment. In this manner the economic situation remains in a continuous condition of change. Indeed, it can scarcely be said that the economic situation as separated from its developmental process has any great significance.

It may be compared to the cross section of a swiftly flowing stream, in which the water rises and falls from time to time, and from time to time overflows or recedes within its banks. If it can be imagined that in addition the current is frequently shifting and eddying from side to side of the channel, then a cross section of the stream at some particular moment may become a fairly good analogy of the economic situation of a given time. A study of unconnected cross sections of our economic world can give no more adequate conception of the forces at work and their probable outcomes than could a similar series of cross sections of the hypothetical stream taken without regard to the conformation of the channel above or below or the amount of fall per mile.

If a concept can be gained of economic situations as developmental phases in the continuous shifting of forces, we shall be much nearer a real understanding of the problem. A shifting flow of forces operating with time as one of its dimensions is a concept which we are not likely to grasp with any degree of assurance, if indeed we are really able to grasp it at all. But the effort will certainly be worthwhile, for the essence of economic activity and the source of the multitude of situations will be found to lie here and not in the mechanistically conceived and isolated cross section.

If an analogy were to be sought for economic readjustments it would more readily be found in biological forms than in mechanical ones. In fact, economics is, in a broad sense, biological. The forces which dominate buying and selling are the forces of human life. The economic organism itself displays many of the characteristics of a growing biological form. Its form is given it by continuously operating forces. Each situation is a developmental phase which will in some degree be outworn after the lapse of a little time. Further, each adjustment in response to changed conditions may be expected to have continuing effects in one direction, or in several, which propagate themselves forward into the future and which,

as in biological organisms, modify the form of that generation of economic institutions as they develop.

This discussion of the nature of the economic processes may seem unduly long. But it is hardly to be expected that successful devices for the measurement or for the anticipation of price or other phenomena could be developed or understood without understanding the general nature of the flow of economic forces and also the environment and the institutions in which they work. With such a survey we are much better able to evaluate both the devices used and the results obtained.

The Development of Forecasting Methods:

The methods that have been used in attempts at forecasting have been many and diverse. But for the most part they can be grouped into a small number of general types. Of these perhaps six may be mentioned as including the greater part of the field. These are, first, forecasting by analogy with earlier periods; second, forecasting by the projection of an existing trend; third, forecasting from the appearance of events or of combinations of events which were observed to stand in earlier periods in certain empirical relationships to the price or other phenomena in question. A fourth method of forecasting has been by the use of mathematical formulae based on quantitative but empirical relationships with related factors. A fifth method is the analysis of the economic situation generally by unquantitative methods and the deduction of the probable outcome in more or less general terms as a result of this study. What approaches another method is the use of a mathematical formula along with an analytical study of the whole situation and the qualification of the results by pointing out the probable effects of influences which it was not possible to express mathematically.

Of the general methods mentioned, the process of forecasting by analogy stands on the weakest ground. This usually consists in comparing the period under consideration with some period in the past in which the prices or other phenomena behaved in an apparently similar manner, and then assuming that the outcome of the present situation will be parallel or very similar to the earlier one. Generally little or no causal relationship is discovered to offer any reason why the outcome in question should be as predicted. The superficial similarity of movement of series is generally the only basis for the forecast. Yet in spite of the lack of demonstrable causal relationship, this method has been carried to the extent of superimposing graphically a series referring to one period on top of the graph of the same series for an earlier period, and then attempting by analogy to forecast the movement of the present series. This was done numerous times after the European war, when price series of the period were plotted along with similar data for the period after the Civil War and forecasts attempted from the resulting graph.

This type of forecast is based on no particular reasoning except the assumption that there have been similar appearing movements of prices in various epochs, and on the unsupported assumption that "history repeats itself." In every attempt at interpreting events or in projecting their consequences into the future there are implied assumptions as to the identity of the influences at work and as to the manner in which these operate in accomplishing their results. The examination of the data at hand involves inferences as to causation and as to the time periods con-

cerned. The dependability of the results rests largely on the choice of alternative inferences. Usually only a few of these are capable of being tested or demonstrated by statistical means. Others must be chosen on the basis of unquantitative observation. Here the need for sound judgment as to practical affairs, and at the same time an ability to penetrate through external appearances to the underlying flow of forces is essential to success. The simple inference that a price will behave as it did in the past certainly cannot serve as the basis for a forecast in an economic world in which no two situations result from exactly similar chains of causation.

A method quite similar to the forecasting by analogy is in the projection of existing trends into the future. This really rests on no more solid basis than the assumption that the price or other series in question will continue to change in the same direction and at the same rate as in the past. Generally, however, a more discreet use of the method has been made. It has seldom been used in unmodified form except in the forecasting of the growth of population or some other similar mass.

There is likelihood of relatively small error if the projection of trend is carried into the future for only a short period. The mass of population already existing will compose the larger part of the predicted number and its inertia lends considerable stability to its growth. Such extrapolations as to the growth of population are usually done by a "curve of growth" formula. But there is no good reason for expecting that a particular type of population will necessarily follow some particular growth curve. Also, new cycles of growth may at any time be initiated by some new discovery in industry or science. The application is therefore safe only for a very limited future period. When an attempt is made to project a logistic curve for some decades or a century into the future the proceeding ceases to have any real or even probable significance.

The third method of forecasting mentioned was from observation of empirical relationships between events. Many of these have been observed and used in an effort to forecast without resorting to mathematical or analytical methods. Thus it has been observed that a rapid rise in interest rates precedes a decline in security prices and a decline in business activity, and that stock prices have generally risen during periods when rates on ninety day loans were lower than on long time high grade bonds.

It has been observed that the rates of activity in certain sensitive industries have forecasting value. The fluctuation in the production of pig iron has been used in several statistical services and in several forecasting attempts. Efforts have been made to demonstrate that stocks and bond prices would reach a high point fourteen months after the preceding low point in the production of pig iron and would reach a low point eighteen months after the high point in a period of increasing pig iron production.

Most of the statistical services make use of what are called business barometers. These are constructed by combining various series which seem particularly sensitive to business changes into a composite which indicates the probable combined effect of the group of influences. The Brookmire Economic Service uses a barometer composed of data on the physical volume of production of basic industries, the ratio of imports to exports, the turnover of bank deposits, and commercial paper rates. The direction of movement of the composite of these factors and the location of the curve with regard to a normal line is interpreted as forecasting the coming

changes in commodity prices. "When the composite of these factors crosses the neutral zone from a favorable to an unfavorable position the upward movement of commodity prices as shown by Bradstreet's Index will, on the average, reach its highest point five months later. The same lag prevails between a favorable crossing of the neutral zone and the bottom of a downward price movement¹."

In the Harvard Economic Service three curves are used. One of these represents factors related to the amount of speculation, a second indicates the activity of business, and the third represents the rates of interest. The forecasting in this case is partly by the direction of movement of each of these three curves with regard to the other two, partly by the amount of movement, and partly by their preceding movements. Thus a fall of the curve representing speculation indicates a coming decline of the curve representing business, and this is particularly true if the curve representing money rates rises at the same time that the speculation curve declines. If the money rate curve falls while the speculation curve rises active business in the near future is indicated. Various other combinations of movements of the curves indicate revival or recession of business after longer periods of time².

It will be seen that the Brookmire and Harvard forecasting devices both assume a sequence of certain types of events in a recurrent business cycle. It is assumed that variations in the supply of credit occur before variations in speculative activities and these before corresponding changes in the rate of business. Both of these services make other analyses to support or verify the indications of the barometric series used. A study of influences not included in the barometers is made and is used to modify the forecasts suggested by the empirical methods. In between the critical junctures indicated by these methods, it is the analytical study of business conditions that affords the means of interpreting current events.

Many other devices are also used. They are founded on bases of various types and with varying degrees of validity, and range all the way from comprehensive measurements of the fluctuations in many lines of business activity down to the simple assumption that a depression must be followed by prosperity, and that prosperity must be followed by a depression. However, it is clear that such means as the two described above, while empirical in form, have a decidedly useful place to fill and that they may rest on a sound analysis of causal relationships.

The Use of Correlation Methods:

The empirical methods just described are adapted to the detection of turning points in business activity and the indication of direction of trends. But they give only indefinite indications of the amount of change to be expected. Naturally the question of how much the rise or fall will be presents itself shortly after the question of direction. For the handling of this problem it is necessary to discover continuously operating series which stand in a more or less definite relationship to the series to be forecast, and whose relationships to that series are susceptible to definite quantitative measurement.

For this purpose economists have borrowed from biologists the methods of correlation³. In the case of biological data the methods of correlation are used to measure the degree of correspondence between variations of biological forms from their norms. The observations to be correlated here are

often pairs of measurements of the same individual, such as length and thickness of stem of the same plant. In economic data, however, no such logical homogeneity of data is possible. The paired measurements to be correlated do not represent different aspects or measurements of the same thing, but at best measurements of different elements in the same situation, as the price of wheat with the price of corn at the same time. Frequently the two factors to be correlated may not pertain to the same time. A time lag may be introduced and a series of prices correlated with some other series of preceding or following months.

The method of correlation is, therefore, not quite at home in economic data. That is, it was not designed for application to data of this type. But the mechanics of the correlation process can be applied to any body of paired observations. The question is not one of feasibility of application, but of logical fitness of the method and of the meaning of the correlation coefficients after they are derived. If sound judgment is used in the selection of the data in the first place and if the results are interpreted carefully afterward, there is no necessary reason why the method should not be made use of here as well as in other fields.

One of the first economists in the United States to use the methods of correlation on economic data was Professor H. L. Moore of Columbia University, who had borrowed it from English economists and from biologists. Professor Moore applied this method in his book, "Economical Cycles: Their Law and Cause," in 1914, and later in "Forecasting the Yield and Price of Cotton" in 1917. The earlier attempts at forecasting were made with a great deal of enthusiasm. Professor Moore's methods and the lessons derived from them are interesting as illustrations of the earlier forecasting by correlation, and as showing how the original assumptions had to be modified before any great progress could be made.

In the book on "Forecasting the Price of Cotton," Moore makes the statement: "By means of the methods presently to be described, it is possible for any person (1) from the current reports of the Weather Bureau as to rainfall and temperature in the states of the Cotton Belt, to forecast the yield of cotton with a greater degree of accuracy than the forecasts of the Department of Agriculture, and (2) from the prospective magnitude of the crop, to forecast the probable price per pound of cotton with a greater precision than the Department of Agriculture forecasts the yield of the crop."

Moore then proceeded by multiple correlation methods to discover the relationships between accumulated rainfall surplus or deficiency and accumulated variations in temperatures and the yield of cotton. A formula was derived whereby yields might be forecast as early as May, but with increasing probability as the season advanced. The method necessarily assumed that the relationships were linear. That is, it was assumed that a deficiency of two inches of rainfall would have just half the influence on the cotton crop of a deficiency of four inches, or a third as much as a deficiency of six inches.

The next step was to discover in the same manner the relationship between the yield of cotton and the general price level on the one hand and the cotton price on the other. From this problem a formula similar to that used in forecasting the yield was derived for the forecasting of the price.

The optimism with which Moore stated his first conclusions was re-

duced later when it came to be realized that not "any person", but only experienced persons with good judgment could be expected to exercise the necessary discrimination in deciding which series to correlate; and that both judgment and experience were much needed afterward in the interpretation of the results of the correlation problem. Also, it soon became clear that the correlation coefficients measured only the degree of association between factors in the past. Thus the obtaining of high coefficients and of a close adherence between the estimates and the actual prices in the past period on which the study was based, were poor criteria of the degree to which the same formula would prove satisfactory in future periods. It was over optimistic to state that "both the yield and the price of cotton, therefore, are so much a matter of routine that they admit of prediction with a high degree of precision."

Yet the method used seemed the only feasible one for the problem at hand. The only way in which future events could reasonably be predicted was by discovering relationships which obtained in the past and which it could reasonably be assumed would continue to obtain in the future. If the forces which made prices or yields could not be determined from past data, or if their influences changed whimsically from year to year the problem was hopeless. A rational analysis of the problem indicated that these basic assumptions were well founded. This being the case, the problem had two aspects. One was the selection of the most significant from among the many series of data concurrent with the prices or yields in question. This was a problem which called for a high degree of sound judgment and a wide acquaintance with the field under investigation. Moore, who himself possessed this qualification amply, overlooked this problem and appeared to assume that it was largely a routine matter requiring no great ability.

The other problem was the development of a method capable of discovering truly or with a close degree of approximation, the relationships which really did exist between the independent series and the series to be forecast. For measuring average relationships between associated factors the method of correlation was the only one at hand and although it was originally devised for a different sort of use, still with reasonable care and particularly with intelligent interpretation of the results there was no reason why it should not do satisfactory service here. Moore deserves considerable credit for adapting the method to economic uses and making known its possibilities.

Shortly after the work done by Moore on cotton yields and prices, the Harvard Committee on Economic Research began an elaborate study of relationships between pairs of economic series, using both the simpler methods of observation and also the correlation method. However, the Harvard Committee proceeded more cautiously than Moore in the discovery of relationships and in their interpretation. Some of the implications of the correlation method as applied to economic data were called in question. A little later it was realized and pointed out that the time influence in such series prevents the consideration of economic variations of this type as random fluctuations which may be expected to assume a normal distribution of each series about its norm. This being the case the standard deviation and the probable error, which in the study of biological data are used to test the dependability of the conclusions, here cease to have any definite

meaning. The only way of testing the results then is by observation and comparison with the actual phenomena as these materialize. Clearly significant mathematical tests are not yet developed.

The Harvard Committee added no new methods in the derivation of forecasting formulae. But it did perform a valuable service in the development of methods usable in the earlier stages of analysis. Improved methods were developed for the discovery of secular and seasonal trends, and in the derivation of simple correlation coefficients and the discovery of lag between time series. The work of Professor Warren M. Persons of the Harvard Committee was highly valuable in the development of these methods and also in amassing a large volume of information regarding characteristics of and relations existing among series related to a large number of our most important industries. The first volume of the Harvard Committee's publication, the *Review of Economic Statistics*, appeared in 1919 with a description of the methods used and an analysis of 24 important series. Others were taken up as rapidly as time permitted. With this information as a background, and by a thoroughgoing technique of analysis, the Harvard Committee has been able to render a valuable and highly dependable service in the interpretation of current economic events.

If the Harvard Committee was chary of the use of multiple correlation methods, others were not so backward. Shortly after 1920 these methods began to be used by various economists in efforts to forecast prices. In 1922 there appeared a study by Dr. Holbrook Working of the University of Minnesota, on "Factors Determining the Price of Potatoes in St. Paul and Minneapolis⁴." In this study a forecasting formula was obtained by using the regression formula from a multiple correlation problem much as was done by Moore in his study of cotton yields and prices. The factors used by Working included the yield per acre, changes in the general price level, and the long time or secular rate of change in the prices resulting from the growing demand for potatoes and the fact that the production increases less rapidly than the demand.

By means of the formula mentioned, estimates of the prices of potatoes were made annually for the crops from 1902 to 1920. In order to obtain estimates of the monthly prices the yearly estimates were multiplied by indexes of seasonal price variation. The average error in the estimates of yearly prices was 9.5 per cent of the estimates. The estimates made in the following years, however, showed wider discrepancies.

In 1923 and 1924 circulars were issued by the New Jersey Agricultural Experiment Station adapting the method and formula of Working to the estimation of the price of New Jersey potatoes and sweet potatoes⁵.

In 1925 there appeared four studies of hog prices by the multiple correlation method. The first one, by G. C. Haas and M. J. B. Ezekiel, developed a formula for forecasting prices of hogs six months in advance by the use of six factors⁶. The second, by Charles F. Sarle, permitted forecasts at three months from four factors⁷. In August of the same year a forecast of hog prices for the coming eleven months was published by Ezekiel in *The Agricultural Situation*, a monthly publication of the Bureau of Agricultural Economics. As the actual prices materialized later they conformed to the general directions of movement forecast, but during five of the months in question deviated from the values of the forecasts by about \$2.00 per hundred pounds, apparently from an inaccurate forecast in one

of the factors used. The fourth hog price study by Sewall Wright involved influences on the corn crop and its prices and on the supply and price of hogs only⁸.

In 1926 the present writer published a study of the prices of beef cattle in which monthly forecasts made for the period from 1922 to 1926, from data available six months in advance, differed from the actual prices by an average of 3.8 per cent of the value of the estimates⁹. In this case, as in most of the others described above, the methods of linear multiple correlation were used. The factors correlated represented related series expressed as percentage deviations from their respective secular trends. The forecast as obtained from the regression formula is therefore expressed as a percentage of the ordinates of seasonal and secular trend projected forward from the period on which the formula was based. This series of forecasts will be discussed more at length as an illustration of a method a few pages farther on.

Curvilinear Correlation Methods:

A shortcoming of the method of multiple linear correlation was soon discovered in the deviations of the forecast from the actual values, which in a good many series showed a tendency to arrange themselves in a more or less definite curve about the straight line of regression. Common sense suggested that many of the relationships between series were of a curvilinear rather than a rectilinear nature. Thus it might be expected that the increases in yields of crops from successive applications of fertilizer would not be at a uniform but at a decreasing rate. A twenty per cent shortage of the corn crop might be expected to have more than twice as much influence on the price as a ten per cent shortage. Similar curvilinear relationships might be expected to appear in nearly any correlation problem. If the correlation method were capable of expressing relationships only in terms of straight lines it might often be expected to yield forecasts wide of the mark or completely misleading.

A method of curvilinear correlation was developed by Mordecai Ezekiel and published in 1924¹⁰. This method involves the computation of the linear multiple correlation regression equation as a first step. From this point on the mathematical devices are largely dispensed with. The deviations of the actual prices from those estimated by the regression equation are then arranged with regard to the magnitude of each independent variable in turn, and are plotted graphically as deviations from the linear regression line. If a curvilinear relationship is present it may be expected to make itself evident by the plotted points assuming a curvilinear arrangement. An approximate curvilinear regression line may be drawn freehand through these points. New values of the estimated price may then be read from this curve and closer approximations to the true curve of regression made from the differences between these second estimates and the true prices.

By this method Ezekiel and others have been able to obtain much higher correlation between the forecasts and the actual prices. This, of course, applied particularly to the period on which the problem was based but the results obtained in forecasting future events were also improved. This method has been used in various correlation problems of the ordinary frequency distribution type. In the estimating or forecasting of prices the

study by Haas and Ezekiel on "Factors Affecting the Price of Hogs," a similar one by F. F. Elliott on "Adjusting Hog Production to Market Demand," and the study on "Factors Related to Lamb Prices," by Ezekiel, are the better known at the present¹¹.

There is little doubt that a method of curvilinear correlation will be the necessary basis of forecasting for most series. There are, of course, some cautions to be observed in its use. In the first place, care is needed in the discovery of the relationships to be sure that the body of data at hand is sufficient to show the true conformation of the curves. This is especially true of the extreme sections of the curves of regression, since there is a strong tendency for the values to be bunched toward the middle of the array with only a few cases at the extremes. In the case of linear correlation this is not so serious because the middle portion of the data is more likely to define the direction of the straight line.

A second caution which applies here somewhat more than in linear correlation is that care should be taken to see that the inflections of the curves of regression are not determined solely by data relating to brief periods of time in which abnormal economic conditions obtain and cause extreme variations in the factors concerned.

What correlation really shows and the ways in which it falls short should be kept in mind both in making the study of relationships between factors and in interpreting the results. The method is adapted simply to measuring the degree to which attributes are associated in a given mass of data. These relationships are discovered by concomitant variations of the values in question as they relate to identical items. That is, if the prices of hogs and of corn are both low or high in the same months and if a rise in one is accompanied by a rise in the other in the same month, some sort of a causal connection is thereby suggested and the degree of relationship is measured by the coefficient of correlation.

Although the presence of correlation (positive or negative) suggests causation it does not by any means establish its presence nor indicate its direction. Thus a perfect correlation would be found between the occurrence of day and of night. As far as the correlation coefficient is concerned it might either be assumed that day causes night or that night causes day, but there is nothing to indicate from the mathematical solution that both are caused by the revolution of the earth. Inference must be used from this point on in reckoning the probable direction and nature of the basic processes of causation.

In the study of economic data we find that we have a network of inter-related forces. In measuring the influences of these we have to use as measurements statistical series showing volumes of production, rates of activity, prices, etc. But each series, such as a price, which is capable of expressing the influences of one of the forces at work is pretty sure to reflect to some degree also the influence of various other forces. Thus the price of corn, which is largely a reflection of the supply of corn available, is also influenced by the number of hogs in the country which constitute to a large degree the demand for corn, as well as various other influences. In many cases, therefore, there arises a perplexing problem in the selection of the series which will best represent the influences it is desired to study, and in the avoidance of series which are too much under influences not perhaps pertinent to the question at hand. If care is not exercised here

it is quite possible that the investigator may obtain spurious results which reflect influences it may be desired to omit, or give a false impression concerning those in question. Here again the satisfactory solution of the problem is seen to depend to a large degree on the amount of judgment possessed by the investigator.

Forecasting by Use of a Demand Curve:

It has been mentioned that there are many influences on prices that either cannot be expressed in quantitative terms or else occur at such rare and irregular intervals that they cannot be used as factors in a correlation problem. Thus some unusual occurrence, such as the outbreak of a war, a flood in a producing section or the outbreak of an epidemic among a class of farm animals, may occasion a change in the supply conditions which could not be handled by the usual methods of correlation, but which would have to be taken into consideration in any worthwhile forecasts of prices.

In most periods of unsettlement it seems likely that the demand condition as expressed in terms of the prevailing price level may be more likely to remain unchanged. This is particularly true if the article in question is some necessary article of food or basic raw material such as the typical farm product. It may be possible to forecast the production or supply of the crop in question by the method of correlation with arbitrary adjustments for the influences not included in the formula, or by various methods of crop forecasting. Then in order to forecast the price it would be necessary to know the relationship between supplies of different sizes and the prices at which these quantities could be sold.

In order to make use of whatever estimate might be made of the coming supply, a few efforts at forecasting have started out with a study of the basic demand conditions. These have attempted to construct a demand curve or schedule of the prices at which each possible quantity of the commodity in question could be sold, after correction is made for variations in the level of prices. An example of this method in forecasting hog prices is explained by Mordecai Ezekiel in the *Journal of the American Statistical Association* for March, 1927¹². Here it was found in comparing forecasts obtained by means of the demand curve with others made by means of a definite formula derived from a correlation study, that the formula gave better results in the test period, but the shortcomings of the demand curve forecasts seemed due to errors in a government forecast of market supplies, which more careful methods in crop forecasting might be expected to correct. The possibilities of the demand curve forecasts thus seem not to be fully exploited as yet. It is likely to prove a valuable means of obtaining comparable figures for checking up on the forecasts made by formula.

The demand curve method has also been used in connection with other commodities. An interesting explanation of its possibilities and a report on the earlier stages of a study involving its use by Holbrook Working in the study of wheat prices is to be found in the July, 1927, issue of the *Journal of Farm Economics*¹³. Here it is pointed out that even for wheat the information as to the probable yields in most countries is often quite inaccurate and that the data on the carryover is subject to considerable error. Until these inaccuracies are largely overcome no method of forecasting could be expected to yield highly accurate forecasts.

There is a further difficulty in the demand curve pointed out by Working in that the exact form of the demand schedule is not known and that up to the present it is only possible to say that such a schedule lies within certain reasonably narrow limits. Further, it cannot be assumed that the schedule would remain fixed in its shape or location even if it could be discovered exactly for a given period of time. Changes in the per capita consumption of wheat, as well as of other products, are clearly occurring constantly. In such staple food products these changes may be assumed to be at a relatively slow rate. But this is not necessarily true of all products, and constant watchfulness would be necessary in the use of this method as well as of others to be sure that the basic relationships have not shifted between the time they were discovered and the time the application is made by their use in a forecast.

Forecasting by Analysis of the Economic Situation:

The fifth method of anticipating economic changes that was mentioned at the beginning of this section was by a careful rationalistic study of the factors of the economic situation together with its background. Again it must be remembered that an economic situation is simply a cross section in a continuous flow of forces. Each element of the situation may be regarded as actually in motion, but in our cross sectional view is caught or photographed in its position at that particular time.

The analysis which is to serve as the basis for some prediction as to future events must uncover each important line of influence at the time as indicated by prices, quantities of production, etc. But this alone will indicate little as to the future. It will be necessary also to have some means of judging from observation of past events what are the normal relationships between the elements of the situation toward which they will eventually gravitate. Even more important for the forecasts of the immediate future and more in conformity with the dynamic or biological concept of economic progress, it will be necessary to discover the direction of change through which each factor has been passing and the rate at which it has been going.

A comprehensive acquaintance with the organization of an industry and the rate at which it is capable of adjusting itself to a given condition will of course be an essential in making any predictions. Next it will be necessary to obtain the fullest possible description of the situation and of the lines of trend which the factors concerned have followed in bringing it about. Statistical information will furnish the basic material with which to work, and will yield measurements of seasonal or longer time trends, the usual relationships between factors, approximate amounts of time which usually elapse between initial stimuli and the various phases of reaction of different factors to them. Inference must be resorted to in respect to the causal relationships between factors in many cases. Unusual elements existing in the present situation must be given consideration and some judgment arrived at as to their probable influences on the course of development in question.

All of this process of analysis will be seen to proceed along no rigidly fixed path. There are no formulae which may be applied, at least in arriving at the final conclusions. The only methods prescribed are those flexible and often indefinite methods of critical scientific procedure in accord-

ance with which any economic study needs to be made. The conclusions will necessarily be stated in rather general terms as to amounts of change. The same body of information might well serve as a basis for widely varying conclusions by different workers, depending on the importance attached to various factors. The conclusions here even more than with other methods must be taken with some regard to the investigator who has formulated them.

On the other hand, the method of analysis has a great advantage in its unlimited flexibility. Being tied down to no limited mechanical method, it is capable, in the hands of a highly competent investigator, of being adapted to any combination of circumstances that may arise. It is not necessary by this method to ignore any line of influence.

Composite Methods:

It has already been implied that each of the methods of forecasting described has its limitations and that some of them are rather serious. If correlation it used it may be found that some important lines of influence are likely to prove unmanageable and must be left out of consideration in this rather mechanical method. If the demand schedule method is used there may be serious uncertainty as to some element of the supply on which the forecast rests, or the location of the demand schedule may be misjudged to some degree. In the use of the method of analysis there is always uncertainty as to the weight that should be given to each factor concerned. Further, unless the situation seems almost identical with various others in the past, the conclusions must be phrased in such indefinite terms that it may often seem to have but little value.

A little reflection, however, will show that a thorough process of analysis is implied in the use of the correlation or in the demand curve method. These are not purely mechanical, but involve a careful study to discover which are the significant factors of causation and how much dependence can be placed in each of them in the explanation and consequently in the forecasting of changes in prices or other phenomena. Before a satisfactory formula can be put into use, it is necessary to examine a large mass of related data to discover the significant series, the degrees of time lag or lead between them, and the closeness of their relationships to the price in question. These series must then be placed in the most significant relationship to the price series and forecasts tried and tested out by comparisons with the actual prices. The differences between the forecasts and the actual prices must be compared to the amount of variation which existed between the original series and its norm, and the reduction in the standard deviation taken as one of the more significant criteria of the success of the effort.

As a result of this process of analysis it may be said that the assumptions and implications on which the forecasting formula rests are given a test which is generally more rigorous than any which could be applied by the unquantitative and less definite methods of a purely rational and unmechanical analysis. Without much doubt the weaknesses of the formula may be expected to come to light. There is therefore an advantage in combining into such a form any factors which are amenable to such methods.

The factors which cannot be expressed quantitatively in a regular and continuous statistical series must be dealt with by the less exact method of rational analysis. There will nearly always be found influences which

cannot be handled by either of the two methods just discussed. But these cannot be ignored because at various times they may grow into forces which dominate the situation. The predictions of the mathematical methods must constantly be qualified in view of this unmathematical process of observation and analysis.

At the same time that forecasts are made by the method of correlation it would be desirable to make parallel predictions by the demand curve method. These may be used as a check on the other series of forecasts. It is likely also that some factors which could not be handled by the correlation method may be taken into account by estimating their probable effects on the size or yield of the crop or other supply and then interpreted into price by means of the demand schedule. We would naturally be prepared to place much more dependence on forecasts arrived at independently by two more or less independent methods than on the forecast by one alone. And where the two disagree we would be prepared to regard each with skepticism and to fall back on rational analysis.

The ideal method of forecasting will thus be a composite of all the methods available, so used as to furnish checks on each other, and to take each factor into consideration by the method most appropriate to it. Further, the results must constantly be compared to the actual prices as they materialize and the methods used revised to eliminate defects, or include new data.

An Example of Price Forecasting:

In Fig. 1 is shown a comparison of two series of forecasts of cattle prices made six months in advance. One is based on a formula obtained by linear correlation, and the other by curvilinear methods. The dotted line, based on linear correlation, will be observed to follow the actual prices closely only for part of the period concerned, and then to indicate the direction of the price movement generally, but not the amount.

Both of these forecasts were based on the same series of factors related to cattle prices. One of these is the price of corn taken seven months before the cattle price in question, since corn is the most important single material used in the fattening of cattle. The typical feeding period is about six months, but the highest correlation is obtained with a seven months lag. This is probably because the farmers who feed cattle are influenced in their decisions by the outlook for corn at a time slightly before they buy the feeder cattle.

A second factor was the margin or difference in price between feeders and fat cattle six months in advance of the cattle price. The amount of premium obtained on the fat cattle over the feeders is second only to the price of corn as a determinant of the profit in cattle feeding. Its highest correlation with cattle prices was obtained at six months.

A third factor was an index of the condition of the forage and pasture crops in the corn belt seven months before the cattle price. The seven months lag seems explainable on the same grounds as in the case of the corn price.

A fourth factor is the condition of the ranges in the western states in which the feeders are raised. This factor showed the closest relationship—an inverse one—ten months in advance of the cattle price. It should be remembered that a change in the condition of the ranges, as from a rain

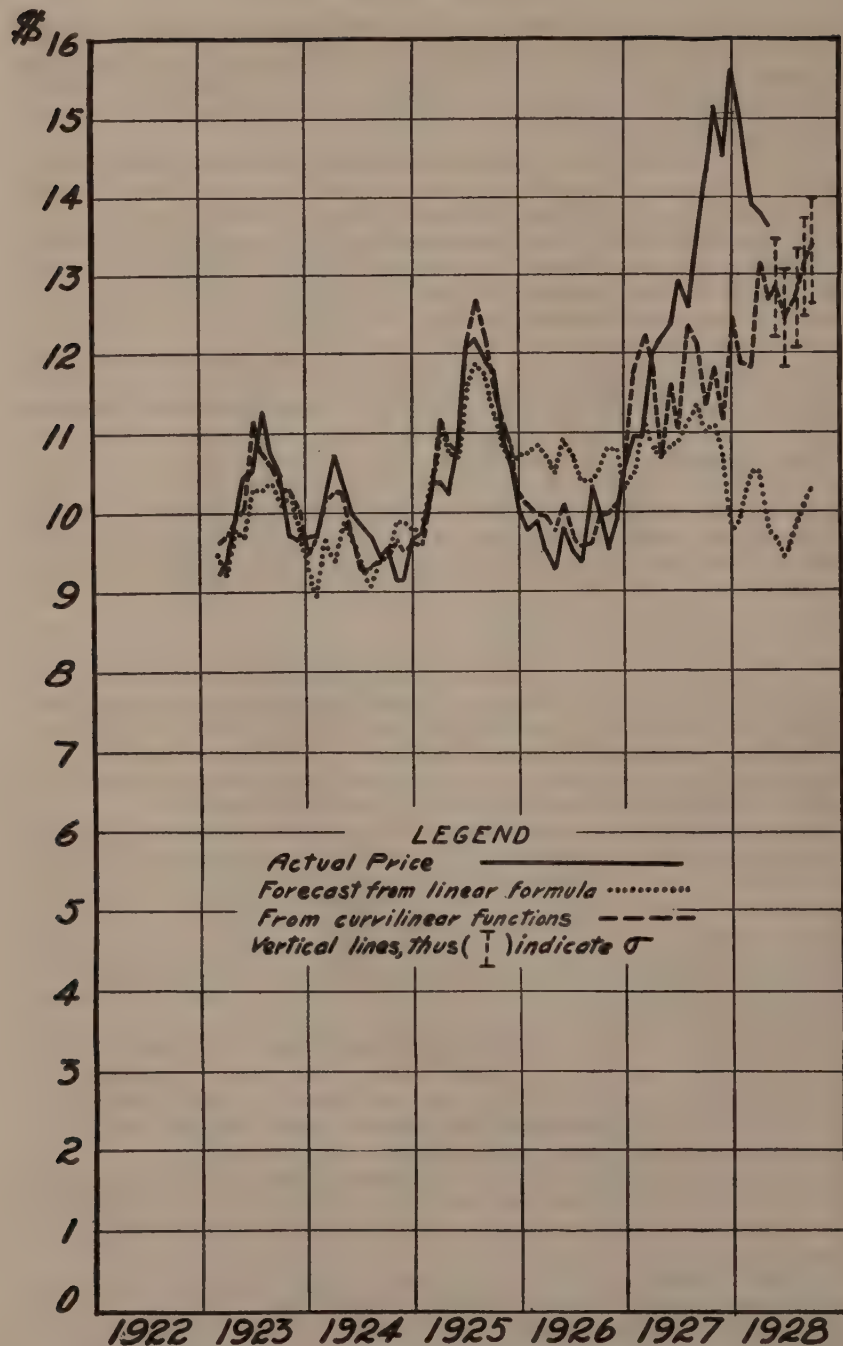


Fig. 1. Cattle prices forecast from data available six months in advance.

or a drought, continued to influence the condition of the western feeders and other cattle for some time and does not reach its maximum effect until a somewhat later date.

The fifth and sixth factors have to do with the shipment of feeder cattle from the stockyards into the feeding sections. One of these consists in the number of feeder cattle shipped per million of population, the figures being taken six months before the cattle price in question. The other is intended to represent the changing number of cattle in the country and consists of the number of feeders shipped during the twelve months ending six months before the price to be forecast.

In discovering the relationships on which forecasts could be based percentage deviations of the factors from their respective averages were put in a multiple correlation equation with the prices of cattle at the periods in the future named. From this equation, or rather from the series of simultaneous equations involved, the following prediction formula was derived:

$$\bar{X} = M_x + .22 i + .40 m - .16 n - .10 p - .52 q.$$

\bar{X} is the price to be forecast. M_x is the mean of the cattle prices in the period on which the study was based. (i), (m), (n), etc., are the percentage deviations from their respective means of the factors concerned, (i) representing the price of corn, (m) the shipments of feeders six months in advance, (n) the shipment of feeders during the year ending six months before, (p) the feeder margin, (q) the condition of the western ranges, and (r) the condition of the forage and pasture crops in the corn belt.

It will be observed that while the forecasts obtained from this formula conform fairly well to the actual prices from 1923 to 1925, they depart from them more and more widely in 1926 and 1927. This seems to be partly because of wide deviations of some of the factors from their means during the later years. The relationships found by the linear correlation method simply represent the average change in the cattle price associated with a unit change in the independent factor. Actually, as is shown in the curve in Fig. 2, a rise of ten cents in the corn price from its average may be associated with a much smaller change in cattle prices than a ten cents rise starting from a point twenty cents higher.

Similar curvilinear relationships are observable in other factors. An increase in the cattle price margin from fifty to sixty per cent would be expected to result in a considerable increase in the amount of feeding. But a change in margin from 80 to 90 per cent would have a much smaller effect because the feeders would realize that such an unusually wide margin represented a purely temporary condition. Therefore, they would not seriously take it into consideration in planning their feeding operations for coming months.

The discovery of these curvilinear relationships by the method of successive approximations described a few pages back, gives us a means of anticipating the effects of more extreme variations in the related factors than could be handled by the simpler linear correlation. These forecasts are made not by means of a formula such as the one given above, but by reading the values of the cattle price directly from the functional curves, such as are shown in Figs. 2 and 3. Thus if the price of corn is 90 cents, we find by interpolation on the functional curve in Fig. 2 that cattle may

RELATIONSHIP OF CORN PRICE TO FAT CATTLE
PRICES SEVEN MONTHS LATER

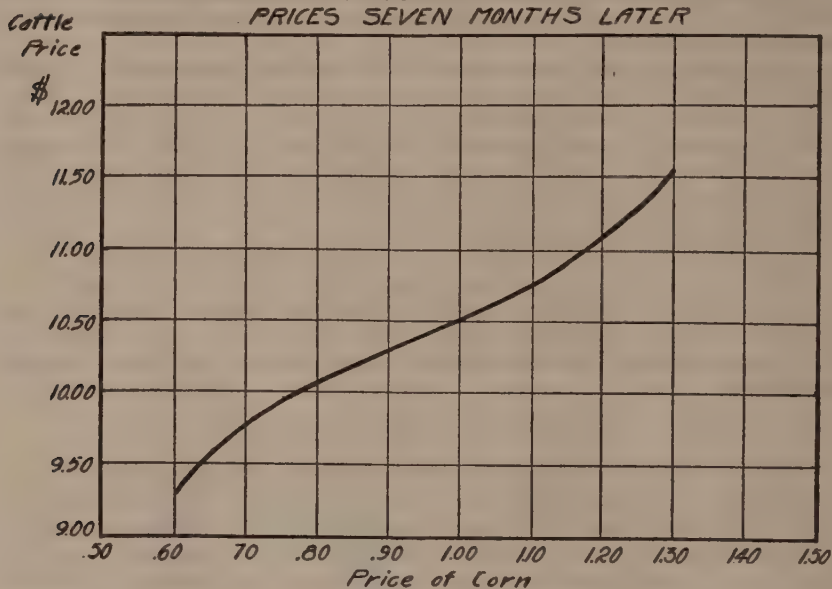


Fig. 2.

RELATIONSHIP OF FEEDER MARGIN TO FAT CATTLE
PRICES SIX MONTHS LATER

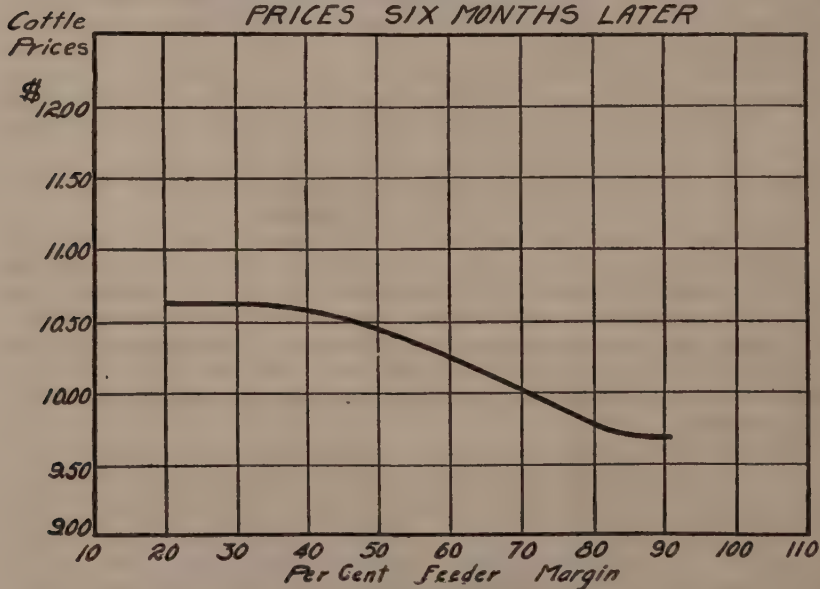


Fig. 3.

be expected to be about \$10.35. If the feeder margin is 50 per cent, we read from the curve shown in Fig. 3 that the price of cattle most often associated with this margin is \$10.45. The influences of the various factors are discovered in this same way and are combined into their composite price influence which is the forecast in question.

The forecasts from the curvilinear relationships will be observed from Fig. 1 to conform much more closely to the actual prices than did those from the linear relationships in 1926 and 1927. A noteworthy discrepancy still exists, however, from the middle of 1927 to the spring of 1928. This evidently reflects the dominance during this period of forces which were not present during the base period, and which might, not improbably, disappear after a short time. Incidentally, the divergences under these conditions may be expected to help in locating the deficiencies of the group of influences used and in this way prove highly valuable.

In this case the cause of the discrepancy seems to be in the fact that in the late spring of 1927 a large number of ranchers and farmers decided, because of the government reports of smaller numbers of cattle in the country, and continuously rising prices, that it was a good time to stock up more heavily on cattle. Consequently a large number of heifers and cows that would otherwise have been sold were kept for breeding purposes. This further shortened the receipts of cattle at the markets and resulted in the prices rising even further until the end of 1927.

A similar influence was observed in the early 1880's, particularly in 1882, when the rapid development of a demand for cattle to stock new range territory in the west, as well as a similar demand for more stock in older sections of the country, caused cattle prices to climb rapidly for about two years. For a few months in 1882 a level of prices was reached much above the years preceding or following. Much the same thing occurred during the late 1890's, particularly in 1899. But clearly these influences operate only at widely separated and irregular periods. They may be explained more by misinformation, or by over response of the farmers to correct information than to any element inherent in the situation itself.

It might be possible to discover factors of a psychological nature associated with these particular reactions on the part of the farmers. But this might be expected to prove very difficult even if data were available covering the periods in which such incidents occurred. In the present case there was no such reaction in the base period of 1923 to the middle of 1927, and no comprehensive data on several of the most important other factors in the earlier periods when these events did occur. Influences not present in the base period could not, of course, be used in forecasting in later years. This would therefore seem to be a case calling for the use of the rational analysis already discussed which should accompany the forecasts by the more mechanical methods.

A person familiar with the cattle industry and with the history of the behavior of cattle prices in past years, might have been expected to modify his forecasts in the summer of 1927. After the reports of a cattle shortage and after the farmers appeared to be holding back more than usual of certain types of breeding animals, it became possible to perceive the drift of the time, and while it would have been difficult to foretell the duration of the abnormally high prices it should have been clear that these were a

matter of only a few months. At the same time, knowledge of the length of time required to increase the supply of cattle at the markets, and reports on the numbers of cattle in the country indicated that levels of prices definitely above those of the preceding years were to be expected for two or three years more.

Application and Interpretation of Forecasts:

It will be noted that the forecasts discussed up to this point have practically all referred to prices. As a matter of fact, it is in the form of probable future levels of price that we may expect forecasts to be most effective in the control of production. Each person operating a business may be expected to modify the plans of that business primarily with the aim of maximizing its profits. Thus a farmer does not reduce his acreage of corn or wheat because he believes that a smaller total crop will be wanted in the country during the coming year, but because he believes that the price and cost situation will make this an unprofitable crop for him. Further, he does not reduce an acreage merely because he fears that it, considered alone, may be unprofitable, but because he thinks that some other crop would prove more profitable or would result in a smaller loss than the one in question.

In any event, the stimulus to increased or decreased activity comes to the individual in the form of a change in prices, either realized or anticipated. With the information that the price of hogs will probably be higher than in the preceding year, the farmer decides to breed a somewhat larger number of sows and to sell less corn in the form of grain. After the pigs are farrowed there are still opportunities for adjustment between the feeding of hogs or the sale or other use of the corn. That is, the pigs may be sold in heavy weights if the price gives promise of keeping up or may be sold in lighter weights if the price declines or threatens to do so. Thus with hogs the opportunity for the use of forecasts is almost continuous throughout the production process.

But with crops the alternative must generally be decided on once and for all before planting. After that step has been taken there is little that can be done short of abandoning part of the acreage. It is true that a promise of high prices is likely to result in giving the crop slightly better care at some stages in its production, but the changes in amount of production from this cause seem decidedly small.

The significance of a forecast should not be forgotten in any effort to make use of such data. The fact that a certain price is predicted for some month does not mean that the forecaster expects the price obtained to be exactly that amount. Rather, it means that out of all the possible figures which might turn out to be the average or typical price in the month in question, this seems to be the most probable one. It must be remembered that as a matter of fact the forecast does not pertain so much to the future as it does to the past. The forecast which is intended to indicate the course of the price or other phenomenon for the future rests on relationships observed between it and related factors in past periods. It is made by applying these relationships to data of the recent past and assuming that they will apply to the near future in the same way as in the past period for which they were discovered.

Permanence of Price Relationships:

Of course it is not necessarily true that the relationships between the factors studied and the price in question will continue to obtain in the future to the same degree as in the past. It may be that the study did not cover all essential influences, or that new ones came into prominence which did not assert themselves in the base period. It might be expected that after the lapse of a considerable period of time, or after some economic cataclysm such as a great war, the price determining factors might be decidedly changed in their relative influences.

With these questions in mind, a price determining formula for fat cattle derived from data for the period 1903 to 1913 was used to estimate prices currently and a similar formula was used to forecast them at two months in the period from 1921 to July, 1925. The results of this experiment, reported in the *Journal of Farm Economics* for October, 1927, shed some interesting light on the permanence of price relationships and on the influence on them of an economic disturbance such as the European War.

"The forecasts made from data available currently were found to differ from the actual prices by an average of 6.7 per cent of the ordinates of secular trend from January, 1921, to July, 1925, the latest data available at that time. . . . But if we average the differences by years we find them to be much larger in the two earlier and less normal years than in the later ones. The average differences are 9.8 per cent for 1921, 9.9 for 1922, 4.8 for 1923, 3.3 for 1924, and 3.5 for the first half of 1925.

"The same method was used in making forecasts at two months. The average difference in this case was 8.8 per cent. But by years the averages were 15.7 per cent for 1921, 11.3 for 1922, 7.2 for 1923, 3.0 for 1924, and 3.3 per cent for the first half of 1925¹⁴."

The changes in these variations seem to give some indication of the degree to which new or unusual combinations of forces dominated the cattle prices in the period just after the war. They also indicate that these unusual influences gradually disappeared during the period named, and that the old forces reestablished themselves and governed prices in pretty much the same manner as in the pre-war years. This was true in spite of some changes in the technique of cattle production and the maturing of cattle at somewhat younger ages than in the pre-war period. An appearance of considerable stability is thus given to price relationships in times reasonably free from powerful abnormal influences. But it is also indicated that in years when such unusual influences are present, resort must be had to other than mechanistic forecasting methods.

Forecasts as Centers of a Belt of Probability:

The forecast does not indicate an exact price for the month named, but rather the center of a range of possible prices within which it appears likely that the average for that month will fall. It is desirable to have some way of designating this range or belt of probability. For this purpose the standard deviation has been used in a few studies¹⁶.

Now the standard deviation, like the correlation coefficient, is not wholly at home in series of time variables. The standard deviation measures a belt on either side of the arithmetic average which includes about sixty-eight per cent of the total number of items. For instance, if one is shooting at a mark and the gun is aimed correctly, but because of variations

in the powder charge, projectile, air currents, etc., the shots are falling scattered in a normal fashion, the standard deviation as measured to either side from the mark will bound a belt within which approximately sixty-eight per cent of the shots fall. Approximately 95 per cent of the cases may be expected to fall within a range of three times the standard deviation on each side of the mark.

The standard deviation, however, rests on the assumption that the items fall in normal manner about the mean value. It cannot be assumed, however, that the items of a time series will fall about an average in the manner of a normal frequency distribution. A time series is not composed of chance variations from a norm, but of a series of unique items each of which depends for its magnitude on the place which it occupies in the sequence.

In periods of time in which no outstandingly abnormal influences are at work, it may be observed that the deviations of a time series from its norm do assume a form essentially that of the normal frequency distribution. In the usual period, therefore, the standard deviation may be used empirically with fairly good results. It is in this empirical manner that it is used in Fig. 1 to indicate the range within which we would normally expect about 68 per cent of the prices to fall during the months from July to October, 1928, for which forecasts are made.

An illustration may make the use of this device more clear. For the month of June, 1928, a forecast of \$12.61 is made as the most probable price for 1200 to 1500 pound steers at Chicago. The standard deviation of the actual prices from the forecasts in the base period was 4.7 per cent of the value of the forecasts. Therefore, if the same influences determine the prices with the same proportionate weights in June, 1928, we would expect that there would be 68 out of 100 chances that the actual price would be within 4.7 per cent of \$12.62, that is, between \$12.03 and \$13.21. In the same manner we would expect that there would be 95 chances out of 100 that the price would fall within a range of three times 4.7 per cent or within \$1.79 of \$12.62. Thus if the same conditions apply as in the period for which the forecasting relationships were discovered, there are 95 chances out of 100 that the price will lie between \$10.83 and \$14.41, and 68 chances that it will be between \$12.03 and \$13.21. When the forecasts are thus viewed in the light of the deviation which experience shows between them and actual prices, they lose much of the rigid and positive appearance with which the popular mind is apt to invest them.

The amount of range permitted by the standard deviation depends largely on the variation in conditions in the base period. This degree of variability determines whether the formula is sufficiently comprehensive and true to life. The width of the standard deviation will also depend on whether it is computed on the deviations between the actual and forecast prices in the base period only, or whether it includes the forecasts made by extrapolation in later months as well. Influences not found in the base period are likely to make themselves felt as time goes on, thus increasing the amount of deviations.

For the illustration given above, a standard deviation was computed from the differences between the actual and forecast prices to include the months from November, 1927, to May, 1928, as well as the base period. This standard deviation is 8.7 per cent of the values of the forecasts as

compared to the 4.7 per cent for the differences in the base period alone. This may be considered as an extreme change in the range and was caused by the pronouncedly unusual influences which dominated the cattle market from the spring of 1927 to the corresponding months in 1928.

As pointed out on an earlier page, such changes in the conformity of the forecast to the actual prices may be expected at irregular but widely separated intervals. When they occur there are two possible courses of action for the research worker. He may either explain them as unusual aberrations which it is not worthwhile to attempt to include in the formula used. Or he may give such explanations as are possible at the time on rational grounds, but rework the correlation problems as soon as feasible and include factors which will reflect the new forces. This latter is the course of action which recommends itself most strongly.

The development of a method of forecasting a price or some other economic phenomenon is not a task that can be carried through to completion in a brief period of time and then regarded as finished beyond the need of further revision. The discovery of the normal relationships not only requires careful research, but, as a practical observation, leaves considerable doubt as to whether the influences found are really comprehensive. Therefore, verification from future developments is necessary. It will usually be found that some adjustment in the method is needed. An old factor may prove unreliable under changed conditions, and the need for new ones may be strongly felt. Constant watchfulness must be exercised to avoid misinterpretation. Frequent revision of the formulae to bring in the most recent data will be needed to make the forecasts most valuable.

It is clear that forecasts of the probable direction of movement of prices may be expected to prove highly valuable to the producers in their efforts to make the most effective and profitable use of their resources. The value to consumers by maintaining a more uniform flow of supply at more nearly constant prices would scarcely be less.

A great amount of research work remains to be done in the discovery of the normal relationships on which the forecasts in each different industry must rest. The degree of success obtained so far gives good promise both of the extension of forecasting methods to other fields and of an increasing accuracy in the industries in which it is already being applied. But there has been a decided change in the outlook of economists interested in forecasting since Moore expressed the opinion that it would be largely a matter of routine which might be done by anyone, once the formula was worked out. This is certainly not the case. A highly developed judgment and a deeply comprehensive acquaintance with an industry is needed both in the development of methods and in interpreting the results from their application. It is safe to say that the development and the interpretation of the forecasts promises to be for a long time a task for the skilled research worker and not for the individual farmer or business man.

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STUDIES IN HOME CANNING*

II. *Indices of Spoilage in Home Canned Foods***

By GERTRUDE SUNDERLIN WITH MAX LEVINE AND P. MABEL NELSON

From the Departments of Bacteriology and Foods and Nutrition.

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INTRODUCTION

In connection with an investigation of the keeping qualities of vegetables and meats canned by the hot water bath method (Sunderlin, Nelson and Levine, 1928), a routine examination was made of each of 2990 jars. These examinations served as bases for detection of spoilage in the canned products. Obviously there must be some criterion or criteria for separating those jars considered spoiled from the others. In this study all jars of canned foods which showed evidence of bacterial growth or changes due to bacterial growth by organoleptic, bacterial or chemical tests were pronounced spoiled. This is a more severe criterion than that used in most of the other reported investigations of spoilage in home canned foods.

HISTORICAL

In studies in home canning of foods, various criteria have been used to detect spoilage.

Biester, Weigley and Knapp (1921), using taste as the criterion, regarded those jars which were considered inedible by the majority of the judges as spoiled. They stated that in several cases the degree of spoilage was so slight that the judges disagreed.

Skinner and Glasgow (1919) made bacteriological examinations of jars of canned asparagus after nine months storage. They made aerobic and anaerobic cultures from both the unspoiled and the spoiled food. They found that jars that were apparently keeping might contain living organisms, and that spore forming aerobes such as those of the *B. subtilis* group, *B. vulgatus* and *B. megatherium* were the types most often found associated with the spoilage of asparagus. They also noted that those jars treated with vinegar (when canned) showed the presence of organisms less frequently than did those not so treated.

Normington (1919) in the examination of home canned peas noted physical appearance, titrated for acidity, made aerobic and anaerobic plate counts as well as direct microscopic counts, and made two sets of gelatin agar shakes, one of which was heated to 80° C., while still liquid, to determine the presence of spores. She later made a chemical analysis of two cans of spoiled canned peas which had been inoculated with "Bacillus A",

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an organism previously isolated from spoiled canned peas. The appearance and odor of the inoculated peas were almost normal, but the peas were found to be softer and more watery on mashing. The juice was cloudy. The taste of the peas was somewhat flat. Gas analyses showed a high percentage of carbon dioxide and hydrogen and a small percentage of oxygen and nitrogen. An increase in acidity was shown by both titration and pH. Creatinin and ammonia were increased in the spoiled peas. She suggested that the determination of creatinin and ammonia, especially the former, may serve to detect bacterial decomposition in canned peas.

Edmondson, Thom and Giltner (1922) noted the condition of the jar and its contents, the hydrogen ion concentration, the types of bacteria present in the juice as determined by Gram stained smears, and types of bacteria found in aerobic and anaerobic cultures. Only part of the jars canned were opened and subjected to these tests. The remaining jars were examined for physical evidence of spoilage such as gas production, foul odor, and disintegration of the material. They stated that "the correlation between these findings and the bacteriological results was so close that for practical purposes the material could be judged as spoiled or not by a physical examination of the jar." They applied the word spoiled to those jars in which there was an active growth of anaerobic bacteria.

The examination of commercially canned foods and the detection of spoilage in foods in general are so closely related to the subject in hand that they are briefly reviewed in the appendix of this paper.

EXPERIMENTAL

METHODS

One jar from each boiler of canned foods was opened as soon as possible after canning and examined bacteriologically and chemically to serve as a basis for comparison later. The jars stored were observed at regular intervals and any showing evidence of spoilage were immediately opened and examined. The remaining jars were opened at the termination of the experiment, after a storage period of five to nine months.

The spoiled jars included 63 of asparagus, 166 of string beans, 49 of Swiss chard, 190 of sweet corn, 26 of tomatoes, 75 of beef and 46 of pork, making a total of 615 jars. These were generally from underprocessed materials, although in many cases other factors such as the use of vegetables which had been held for several days and storage of the processed materials at high temperatures exerted a determining effect.

The observations and tests made on each jar in the routine examination were as follows: appearance, suction, odor, pH, titrable acidity, amino N and ammonia as shown by Sörenson formol titration, microscopic examination of the sediment, bacterial counts on plates at 37° and 20° C., and in dextrose broth tubes. Under appearance were included gas production as evidenced by bubble formation, bulged caps or broken seals; cloudiness of the liquor; sediment; color of the product; consistency and disintegration; and formation of patches of growth.

Suction or vacuum was determined by quickly pulling the rubber from beneath the Mason cap with a pair of pliers. A sharp sound due to the inrush of air was taken to indicate a satisfactory vacuum. A spurting of the liquid from the jar indicated pressure, while an absence of sound was recorded as "no suction". When a perfect seal was secured during the

canning process the new Mason caps were drawn in by suction (due to partial vacuum) as the products cooled. Any raising or bulging of the caps was indicative of pressure.

As quickly as possible after the cap was removed, the odor of the product was noted. Any apparent abnormality was recorded.

The pH of the liquid in the jars was determined colorimetrically. Phenol red, bromthymol blue, chlorphenol red, bromcresol green, methyl red and bromphenol blue, were the indicators found most suitable.

Total acidity was determined by titrating 10 c.c. of the liquid, which had been diluted with 10 c.c. of boiled neutralized distilled water containing 1% phenolphthalein, against N/20 NaOH. The figure obtained divided by two gave the percentage of normal acid.

The liquid which had been neutralized by the titration for total acidity was used for the amino nitrogen and ammonia determinations. (Sørensen formol titration.) To this liquid was added 10 c.c. of 50% neutral formaldehyde which contained 1% phenolphthalein. It was then titrated with N/20 NaOH, and multiplied by the factor 70 to obtain the number of milligrams ammonia and amino nitrogen per liter.

Gram stains were made of the sediment in the jars, obtained by means of a sterile pipette. In the microscopic examination of the stained mounts, the relative number and morphology of the organisms and the presence of spores were noted.

Bacterial counts were determined at 20° and 37° C. on Bacto nutrient agar. In the case of tomatoes, tomato agar was used for plating. Special media made from beans and corn were used at first for the growth of organisms from the spoiled beans and corn, but no advantage was observed.

Dextrose broth with Andrade's indicator in Durham fermentation tubes was employed for evidence of acid and gas production and to allow for growth of some organisms which would not grow on aerobic plates. The tubes were heated for 10 minutes in boiling water and quickly cooled before transfers of 1 c.c. of the liquid from the jars were made into them. The presence and character of growth and acid and gas production were noted after two days and again after one week at 37° C.

In determining which jars of vegetables and meats were spoiled the following criteria were taken as positive evidence of spoilage:

- (1) any marked change in appearance.
- (2) any change in odor.
- (3) a broken seal, bulged cap or pressure on opening.
- (4) a difference in pH of 0.2 or more as compared with the normal jars of the same series.
- (5) a difference of 1 c.c. or more in titrable acidity or formol titration as compared with normal jars of the same series.
- (6) numerous organisms showing in the stain of the sediment.
- (7) excessive counts as indicated by the plate or dextrose broth count.

In assembling and studying the data the indices of spoilage were grouped under three general heads:

- (1) physical evidence of spoilage (organoleptic tests), including appearance, odor and suction (vacuum) changes.
- (2) chemical evidence of spoilage, including change in pH, titrable acidity, and formol titration.

- (3) bacteriological evidence of spoilage, including increase in the numbers of organisms in the stain of the sediment or the living organisms in plate counts or dextrose broth.

RESULTS

Relation between physical, chemical and bacteriological indices of spoilage. The interrelationships between the physical, chemical, and bacteriological tests used to detect spoilage in 615 jars of vegetables and meats classed as spoiled are summarized in Table I. In 200 (32.5%) of the 615 jars of spoiled food examined, there was an absolute correlation between the physical, chemical and bacteriological indices of spoilage. In the different products examined, the proportion showing perfect correlations varied from less than 20% in the case of Swiss chard to more than 50% in the case of asparagus.

In 106 (17.2%) of the 615 spoiled jars there was evidence of spoilage by physical tests, but by neither the chemical nor bacteriological tests used. Of the spoiled chard 55% fell into this group, whereas, in only one jar (3.8%) of the tomatoes was the evidence of spoilage restricted to the physical tests.

TABLE I. THE NUMBER AND PERCENTAGE OF JARS SHOWING EVIDENCE OF SPOILAGE BY PHYSICAL, CHEMICAL, AND BACTERIOLOGICAL TESTS.

Indices of spoilage			Asp'agus 63 jars	Beans 166 jars	Chard 49 jars	Corn 190 jars	Tomat's 26 jars	Beef 75 jars	Pork 46 jars	All prods. 615 jars
†Py.	Ch.	Ba.								
+	+	+	33 (52.4)*	36 (21.7)	9 (18.4)	68 (35.8)	7 (26.9)	28 (37.3)	19 (41.3)	200 (32.5)
+	+	—	7 (11.1)	41 (24.7)	27 (55.1)	14 (7.4)	1 (3.8)	15 (20.0)	1 (2.2)	106 (17.2)
+	—	—	4 (6.3)	32 (19.3)	5 (10.2)	19 (10.0)	0 (0)	1 (1.3)	4 (8.7)	65 (10.6)
+	—	+	11 (17.5)	46 (27.7)	8 (16.3)	16 (8.4)	15 (57.7)	29 (38.7)	3 (6.5)	128 (20.8)
—	+	+	6 (9.5)	1 (0.6)	0 (0)	13 (6.8)	0 (0)	1 (1.3)	8 (17.4)	29 (4.7)
—	—	+	2 (3.2)	6 (3.6)	0 (0)	57 (30.0)	3 (11.5)	0 (0)	4 (8.7)	72 (11.8)
—	+	—	0 (0)	4 (2.4)	0 (0)	3 (1.6)	0 (0)	1 (1.3)	7 (15.2)	15 (2.4)
+			55 (87.3)	155 (93.4)	49 (100)	117 (61.6)	23 (88.5)	73 (97.3)	27 (58.7)	499 (81.1)
+			43 (68.3)	73 (44.0)	14 (28.6)	103 (54.2)	7 (26.9)	31 (41.3)	38 (82.6)	309 (50.2)
+			52 (82.5)	89 (59.6)	17 (34.7)	154 (81.1)	25 (96.2)	58 (77.3)	34 (73.9)	429 (69.7)

* Numbers in parentheses indicate %.

Other numbers indicate number of jars showing positive evidence of spoilage.

†Py.—Physical Ch.—Chemical Ba.—Bacteriological.

In 65 (10.6%) of the 615 jars spoilage was evidenced by both the physical and chemical, but not by the bacteriological tests employed. Spoiled beans and corn were particularly likely to be found in this group. Thus 32 of the 65 jars in this group were beans and 19 corn.

In 128 (20.8%) jars of spoiled products the physical and bacteriological tests only were positive. There was considerable variation in the proportion of the different products falling in this group, ranging from 6.5% in the case of pork to 57.7% for tomatoes.

Twenty-nine (4.7%) of the 615 jars showed no spoilage by physical means, but did give positive evidence by both the chemical and bacteriological tests used. In general the proportion of jars falling in this group was small, ranging from practically none in chard, beans, tomatoes, and beef to 17.4% for pork.

Seventy-two of the 615 jars gave evidence of spoilage by bacteriological tests only. Fifty-seven of these were of corn; these constituted 30% of all of the spoiled corn. The physical tests, if employed alone, are apparently more likely to be misleading in the case of corn than in any of the other products studied. Very few jars of other products, aside from corn, were judged spoiled on the bacteriological basis only.

Only 15 (2.4%) of the 615 jars of spoiled food showed chemical evidence of spoilage without showing either physical or bacteriological evidence. Seven of these jars were underprocessed sausage where the only apparent abnormality was a decrease in pH. The other products showed none or very low percentages of spoilage evidenced by chemical tests alone.

In this series of observations, if we had depended on the physical tests alone as the criterion of spoilage, 116 of the 615 jars called spoiled would have been missed. The physical tests used did not include taste, and it is probable that an "off" flavor would have been apparent in many of these 116 jars, especially in those where there was a change in pH or acidity, as in the corn and the pork. (Taste was purposely omitted from the criteria, because it is felt that it is undesirable and unwise to test canned foods for spoilage by actually bringing them in contact with the mucous membranes of the mouth.) Spoilage was evidenced by physical tests in 499 jars (81.1%), by chemical tests in 309 jars (50.2%), and by bacteriological tests in 429 jars (69.7%). In general, physical evidence was a good criterion of spoilage in asparagus, beans, chard, tomato and beef, but not as good in corn and pork. Chemical evidence of spoilage showed up in 82.6% of the cans of spoiled pork, but in less than 50% of most of the products. Bacteriological evidence of spoilage was detected in only 34.7% of the spoiled chard, but in over 59% of each of the other products.

Of all the jars showing spoilage by the physical tests used (499 jars), only 66% showed evidence by the bacteriological, and 53% by the chemical tests. Of those showing spoilage by the chemical tests (309 jars), 86% showed physical signs of spoilage and 74% bacteriological. Of those showing bacteriological evidence of spoilage (429 jars), 76% showed physical evidence and 53% chemical. These relations are brought out in Table II.

TABLE II. THE RELATIONS OF PHYSICAL, CHEMICAL AND BACTERIOLOGICAL INDICES OF SPOILAGE.

Series	Positive evidence of spoilage	Number of jars	Percentage of each series showing spoilage by tests employed		
			Physical	Chemical	Bacteriological
I	Physical	499	100%	53%	66%
II	Chemical	309	86%	100%	74%
III	Bacteriological	429	76%	53%	100%

It appears from a consideration of the data in Tables I and II, that while physical evidence is the best single index of spoilage, it would be necessary to employ both chemical and bacteriological tests as well, if it is desired to detect all cases of spoilage.

Relative value of various determinations. A summary of the incidence of spoilage observed by the various tests employed is given in Table III. Of the different observations classed under physical evidence of spoilage (odor, appearance and suction), the odor of the product was better as an

index of spoilage than appearance or suction change, except in the case of spoiled beef, where appearance was altered in more jars than was the odor. Appearance was a good index of spoilage in beans, chard and beef, but not so dependable in the other products. A change in suction was evident in 57.7% of the jars of tomatoes, but in less than 38% of the jars of any of the other materials.

The chemical tests used for the detection of spoilage (total acidity, pH and formol titration) varied greatly in their value with the different food products. The formol titration, as would be expected, was of no value as an index of spoilage in the vegetable products. There was a decided change in formol titration in beef and pork when decomposition was advanced, but this test was of no value in detecting incipient decomposition. A significant difference in formol titration was observed in only 26-29% of the samples of beef and pork which had spoiled.

The reaction as indicated by pH was found to be more suitable than the titrable acidity as an index of change in reaction in the case of asparagus, beans, chard and pork, but the reverse seemed to be true in the case of corn, beef and tomatoes.

The presence of organisms capable of growing in dextrose broth tubes was a more frequent index of spoilage than the other bacteriological tests used in the case of chard, corn, beef and pork; but in asparagus, tomatoes and beans the appearance of numerous organisms in the microscopic examination of the sediment was the most frequent bacteriological index of spoilage. In spoilage caused by bacteria, one might expect to invariably be able either to see or isolate the organisms responsible. The experience encountered in this study was that in 30% of the jars judged spoiled, organisms were not observed either by staining methods or by attempts at cultivation. This is in line with the experience of Bitting and Bitting (1917), who found that the length of time the organisms live in cans may vary from a few days to seven years, and that the bacteria may disintegrate so that they do not even show in the stain. Weinzirl (1919) also found that 6 of 20 "hard swells" failed to show organisms. The difficulty of differentiating bacteria from amorphous matter in the stains of some products like corn, the possibility of the organisms developing and dying within a few days and the likelihood of autolytic enzymes disintegrating the bacteria, as well as the chance of having organisms present which will not grow under the conditions used for cultivating them, all combine to make negative bacteriological evidence of spoilage unreliable. On the other hand, the fact that there may be living organisms (spores) in canned foods which are not considered spoiled complicates the interpretation of results. The necessity for drawing a line somewhere, in the interpretation of the significance of the bacterial counts obtained from the products tested, led us arbitrarily to consider a count of more than 25 living organisms per c.c. as evidence of probable spoilage.

In general, of the different indices of spoilage used in this series of observations, odor ranked highest. In all the products but corn and pork, the odor was altered in over 70% of those jars classed as spoiled. Considering all of the spoiled samples, change in odor was evident in 68.3% of the jars. This was more than 20% above what was obtained with any other test employed. The growth of organisms in dextrose broth tubes, presence of organisms in the stain, change in appearance, titrable acidity and pH,

TABLE III. INCIDENCE OF SPOILAGE OBSERVED BY VARIOUS PHYSICAL, CHEMICAL AND BACTERIOLOGICAL TESTS.

Index of Spoilage	Asparagus 63 jars	Beans 166 jars	Chard 49 jars	Corn 190 jars	Tomatoes 26 jars	Beef 75 jars	Pork 46 jars	Total 615 jars
Change in appearance	27 (42.9)*	89 (59.6)	34 (69.4)	32 (16.8)	10 (38.5)	64 (85.3)	14 (30.4)	270 (43.9)
Change in suction	17 (27.0)	42 (25.3)	15 (30.6)	51 (57.8)	15 (57.7)	28 (37.3)	15 (32.6)	183 (29.8)
Change in odor	49 (77.8)	134 (80.7)	36 (73.5)	99 (52.1)	23 (88.5)	55 (73.3)	24 (52.2)	420 (68.3)
Change in pH	39 (61.9)	56 (33.7)	13 (26.5)	91 (47.9)	4 (15.4)	7 (9.3)	33 (71.7)	243 (39.5)
Change in titrable acidity	28 (44.4)	47 (28.3)	8 (16.3)	122 (64.2)	6 (23.1)	26 (34.7)	17 (37.0)	254 (41.3)
Change in formal titration	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	20 (26.7)	13 (28.3)	33 (5.4)
More organisms in stain	49 (77.8)	65 (39.2)	8 (16.3)	88 (46.3)	25 (96.2)	33 (43.4)	17 (37.0)	285 (46.3)
More organisms in aerobic count	9 (14.3)	29 (17.5)	10 (20.4)	95 (50.0)	12 (46.2)	49 (65.4)	29 (63.0)	233 (37.9)
Growth in dextrose broth tubes	17 (27.0)	42 (25.3)	12 (24.5)	123 (64.7)	16 (61.5)	54 (72.0)	32 (69.0)	296 (48.1)

* Numbers in parentheses indicate percentages.

Other figures indicate number of jars showing positive evidence of spoilage.

and organisms in aerobic plate counts ranked in the given order, ranging from 48.1% to 37.9% of the jars spoiled; while the suction change and formol titration variation ranked lowest as indices of spoilage.

Relation between change in titrable acidity and pH. In many instances, there was no apparent correlation between change in titrable acidity and pH. In most products the pH of different jars from the same batch of material varied within a range of less than 0.3, whereas the titrable acidity varied through a wider range (1 to 4% normal acid). In view of the fact that the actual reaction of any given jar could not be ascertained, it was not possible to detect a change in reaction unless the change was considerable and sufficient to bring the reaction well beyond the observed range of the unspoiled samples. Thus if the unspoiled samples showed a reaction range of 1 to 4% normal acid it would be impossible to say that a spoiled jar showing 3.8% normal acid had not increased in acidity, for it might have contained but 1% initially. This was a difficulty encountered in interpreting titrable acidities.

The titrable acidity was significantly increased without a corresponding change in pH in eleven jars of spoiled corn, twenty jars of spoiled beef and five jars of spoiled pork. The pH decreased while the titrable acidity was not significantly beyond the range of reaction for unspoiled jars in ten jars of spoiled corn, seventeen jars of spoiled pork and one jar of spoiled beef. In four jars of spoiled beef there was a decrease in acidity as determined by hydrogen ion concentration accompanied by an increase in titrable acidity.

This lack of correlation between titrable acidity and hydrogen ion concentration may be explained on the basis of various types of organisms growing in buffered mediums and producing end products which have different reactions, dissociation constants, and buffer values.

In the spoiled beans and asparagus it was observed that the change in hydrogen ion concentration was well correlated with that of titrable acidity.

TABLE IV. RELATION OF FORMOL TITRATION TO SPOILAGE IN BEEF AND PORK.

Formol titration range cc N/20 NaOH for 10 cc. liquor.	Beef		Pork	
	Unspoiled	Spoiled	Unspoiled	Spoiled
	Number of jars		Number of jars	
0 - 4				1
4.1 - 8		3	2	0
8.1 - 12	156	47	12	3
12.1 - 16	53	8	160	31
16.1 - 20	14	4	8	5
20.1 - 24				0
24.1 - 28				2
28.1 - 32		1		2
32.1 - 36				1
36.1 - 40		3		1
40.1 - 44		2		
44.1 - 48		1		
48.1 - 52		2		
60.1 - 64		1		
64.1 - 68		1		
68.1 - 72		1		
96.1 - 100		1		
Total	223	75	182	46

The formol titration in beef and pork as an index of spoilage. It is evident from a comparative study of the figures for the formol titration of the spoiled and the unspoiled beef and pork that while a decided increase in formol titration indicates spoilage, a titration within the normal range does not necessarily point to the absence of spoilage. Table IV gives the distribution of formol titration results from both the spoiled and the unspoiled products. In many of the spoiled jars there was a marked increase in formol titration; on the other hand, in many jars of meat showing spoilage or incipient decomposition the formol titration did not vary from that of the unspoiled meat.

That a decided increase in amino nitrogen and NH_3 (formol titration) was closely related to advanced decomposition and that formol titration was not an aid in detecting incipient decomposition were evident from a study of the data. The relation between increase in formol titration and odor as observed with 121 jars of spoiled beef and pork is shown in Table V.

TABLE V. THE RELATION OF FORMOL TITRATION AND ODOR IN SPOILED MEAT.

Odor	No. of jars	Formol titration	
		Number showing distinct deviation from average of unspoiled material	Average increase over unspoiled jars (Mg.N per liter).
No change	42	4	7
Slightly off	36	2	0
Distinctly off or putrid	43	27	980

It was found that the jars of beef and pork which showed decided physical evidence of spoilage, particularly disintegration, and were consequently opened before the end of the experiment (19 days to 5 months) showed, as a rule, a much greater increase in formol titration than those jars in which physical evidence of spoilage was not marked, and which, in consequence, were stored for the entire duration of the experiment (8-9 months). This relationship is brought out by the figures given in Table VI.

TABLE VI. THE RELATION OF FORMOL TITRATION AND PHYSICAL EVIDENCE OF SPOILAGE.

	No. of jars	Formol titration	
		Number showing distinct deviation from average of unspoiled material.	Average increase over unspoiled jars (Mg.N per liter).
Jars opened because of distinct evidence of spoilage	45	27	860.0
Jars opened at close of experiment	76	6	4.9

The relation between increase in acidity and informol titration in spoiled meat. There was a correlation between the change in total acidity and the change in formol titration in the jars of spoiled beef and pork. This is shown in Table VII.

TABLE VII. RELATION BETWEEN INCREASE
IN ACIDITY AND IN FORMOL TITRATION IN 121
JARS OF SPOILED MEAT

Number of jars	Average deviation from unspoiled meat	
	Titration acidity*	Formol titration†
3	-80	7.7
78	0	5.6
16	52	154.0
12	138	700.0
8	258	2282.0
4	482	1890.0

* In terms of cc. normal NaOH to neutralize 1 liter of sample.

† Expressed in mg.N per liter.

In the 78 jars of spoiled meat where the total acidity was similar to the unspoiled meat, there was almost no change in formol titration. In the 16 jars that showed an increase in acidity of 52 c.c. N acid per liter there was a corresponding increase of 154 mg. per liter in amino nitrogen and ammonia (formol titration). In the 12 jars that showed an increased acidity of 138 c.c. there was an increase of 700 mg. N in the formol titration. In the 8 jars that showed an increase of 258 c.c. n acidity the formol titration increased to the extent of 222 mg. amino nitrogen and ammonia per liter. These figures show a positive correlation between increase in titrable acidity and in the development of amino nitrogen and ammonia as determined by the formol titration.

A heat resistant anaerobic Actinomyces found in spoiled beans. Actinomycete-like forms were observed in the stain of the sediment of 30 jars of the spoiled string beans, which had been processed from one to four hours in the boiling water bath. Spoilage of canned products due to *Actinomyces* was not anticipated, as the thermal death time of the *Actinomyces* spores is usually given as not longer than one hour at 80° or 90° C. Bergey (1919), however, noted two thread-like thermophilic organisms which required two hours at 100° C. for killing. It is also true that most species of the genus *Actinomyces* are aerobic forms and thus would not grow in the sealed jars. Of the 64 species of *Actinomyces* listed in Bergey's Manual of Determinative Bacteriology (1923), only one is described as anaerobic and three as microaerophilic.

The beans in which the actinomycete-like forms were observed had been either underprocessed, kept one or more days after gathering before canning, or incubated at 37° C.

In two of the jars incubated at 37° C., a clump of *Actinomyces* growth as large as a pea was visible on a bean submerged in the liquid. This growth was cream, pink and white in color and consisted of smooth, compact nodules forming a mass with an irregular surface.

That the organisms grew anaerobically or at least under partial anaerobic conditions in the jars was shown by the evidence of suction as the jars were opened.

In view of the fact that the center of the jar of beans processed in a boiling water bath reaches the boiling temperature in less than one hour,

it seems that in the two jars processed for four hours the organisms resisted the boiling temperature for over three hours. Of the remaining jars showing these organisms, seven had been boiled for three hours, seven for two and one-half hours, eight for two hours, and six for one hour.

Transfers were made from the two jars showing nodular growths into thirteen different kinds of media, including the standard culture media as well as bean agar, beans in large tubes, carrot plugs, glycerin agar, potato agar, casein digest agar, and 2% milk agar. The media were incubated at 37° C. under aerobic and anaerobic conditions.

The only growth which resembled the original clump was secured after an incubation period of five weeks in a Novy jar in which the air had been displaced with hydrogen, at 37° C. One colony was observed on a veal infusion agar plate. A stain of this colony showed the actinomycete-like organisms, and transfers were made into veal infusion agar and bean agar on plates and tubes and into sterilized string beans in large tubes. After one month in a Novy jar at 37° C., a small clump developed in one tube of beans. A stain of this showed the characteristic structures of the *Actinomycetes*.

Due to the length of time it takes for visible growth to occur and the irregularity of occurrence of growth in the tubes inoculated, a detailed study of the cultural characteristics has not been possible.

SUMMARY AND CONCLUSIONS

1. Data regarding the indices of spoilage in 615 jars of spoiled canned foods are presented.

2. The criteria used for judging spoilage are described. These included physical tests such as appearance, odor and suction; chemical tests for total acidity, pH and the formol titration; and bacteriological tests such as microscopic examination of the sediment, plate count at 37 and 20° C., and growth in dextrose fermentation tubes.

3. Physical evidences were more frequent indices of spoilage than either the chemical or bacteriological tests used. No one group of tests was sufficient to detect all cases of spoilage. It was necessary to employ physical, chemical and bacteriological tests to detect all spoilage.

4. Of the different tests used, change in odor ranked highest with respect to the detection of spoilage. Change in suction and in formol titration ranked lowest.

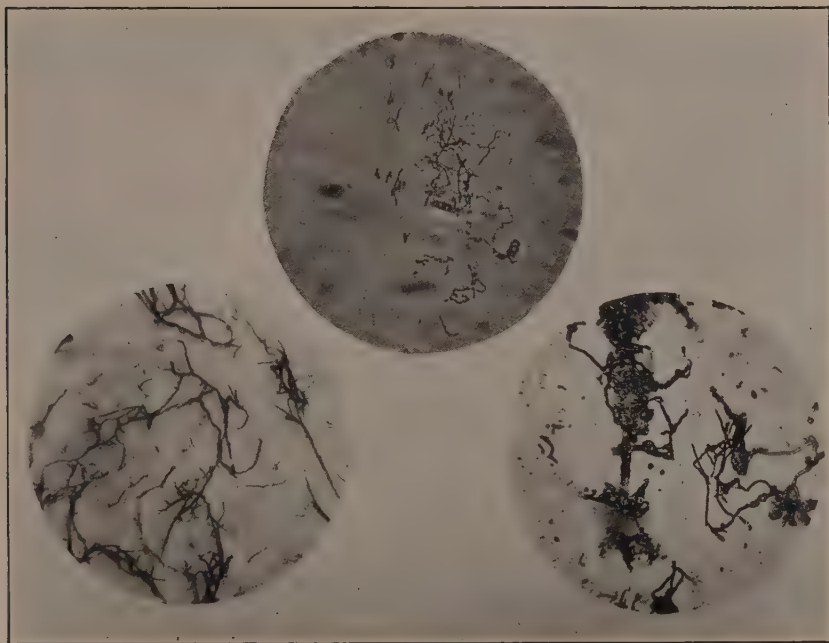
5. In spoiled beans and asparagus, change in hydrogen ion concentration was well correlated with change in titrable acidity. In spoiled beef, pork and corn, there was no apparent correlation between change in titrable acidity and in hydrogen ion concentration.

6. A decided increase in formol titration indicated spoilage in beef and pork, but a titration within the normal range did not necessarily indicate absence of spoilage. That the increase in formol titration was an index of advanced decomposition of meat was shown by the correlation of increase in this determination with pronounced odor and with distinct physical evidence of spoilage. Formol titration, however, was not suitable as an index of slight or incipient spoilage.

7. There was a correlation between increase in titrable acidity and increase in formol titration in the spoiled meat.

Stained Mounts of *Actinomyces* from Jars of Spoiled Beans.

FIGURE I



8. A heat resistant anaerobic *Actinomyces* was found in jars of spoiled beans.

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APPENDIX

The following is a condensed review of part of the literature upon the examination of spoiled canned foods and the detection of spoilage in foods:

- 1911 Bacon and Dunbar (1911) reported that canned tomatoes, when sound, contain no volatile acids, but a considerable amount of citric acid and invert sugar, while when spoiled they contain quite large amounts of volatile acids and little or no invert sugar or citric acid. Howard (1911) described a method for microscopic examination of fruits and tomatoes for detection of molds, yeast and bacteria, and disintegration of the plant cells.
- 1915 Schneider (1915) outlined the micro-analytical and bacteriological methods in the food and drug laboratories and suggested using Eber's test to detect putrefactive change in meat. The test as described appears to be a qualitative test for NH_3 .
- 1916 Bigelow (1916) outlined the methods employed by technical laboratories in the inspection of canned foods. These methods included external appearance of container; odor, flavor and appearance of contents; microscopical and bacteriological examinations; determination of acidity; and the composition of the gas in cans with bulged ends.
- Billings (1916) stated that canned goods may manifest their unsanitary condition by becoming swelled due to the development of gas within the can, or by sourness or putrefaction, or the presence of living or dead micro-organisms in excessive numbers.
- Tillmans and Mildner (1916) investigated chemical means of detection of incipient decomposition in meat. The determination of ammonia and of amino nitrogen gave positive results only when the meat had reached an advanced stage of putrefaction. They devised a method for judging the condition of the meat by the amount of dissolved oxygen remaining in an aqueous extract after various periods of incubation. If the oxygen had disappeared within four hours after incubation the food was judged unfit for use.
- 1917 Bitting and Bitting (1917) described a method for determining spoilage and potential spoilage in packs of canned goods. They recommended the study of the history of the foods in question; selection of representative cans; observation of gross appearance; incubation of cans at 37 and 55° C. for one to fifteen days; use of a vacuum gauge to determine whether a vacuum is or is not present; testing of the gas in swelled cans for hydrogen; inspection of the contents as to color, texture, consistency, odor and flavor and for small infected spots; inspection of the can for rust spots, black patches and erosion; examination of the unstained liquid microscopically for actively motile organisms; and the inoculation of culture media under aerobic and anaerobic conditions in all cases of doubt.
- Bushnell and Utt (1917) examined 52 samples of various brands of canned salmon for bacteria. They used dextrose fermentation tubes, agar plates, agar shakes, milk, and Endo agar with negative results.

Howard, Burton and Stevenson (1917) reported the results of a five-year investigation to establish a basis for judging tomato products. In their experience, tomato products from stock judged acceptable by visual inspection never showed high counts of micro-organisms, while products from stock which was not good or was improperly handled showed high counts.

- 1919 Brauer (1919) stated that incipient decomposition in sausage and canned foods can be detected by inoculating dextrose bouillon with a small portion of the sample, incubating at 38° C. for 24-48 hours, and noting whether or not gas is produced during this period. This method was based on the opinion that all bacteria which are harmful to health, when they grow in food, ferment dextrose broth with gas production.

Falk, Baumann and McGuire (1919) tested decomposing meat for total nitrogen, ammonia, creatine and creatinine and purine nitrogen. Different strains of organisms showed marked differences as well as similarities in changes. The purine values decreased rapidly with some organisms, and distinctly increased with others. Ammonia increased in every case and they suggested that this might be of value in determining when the meat becomes unsuitable for use. Falk and McGuire (1919) determined the ammonia content of meat undergoing decomposition. Meat that decomposed at room temperature containing 0.3 to 0.4 mg. ammonia nitrogen per gram of meat was unfit for food, while that decomposed at 0 to 5° C. might contain as high as 3.0 mg. ammonia nitrogen per gram before the meat was unfit to eat.

Hunter and Thom (1919) emphasized the fact that "sterility is not to be confused with fitness for food." Cultures from 350 cans of salmon showed 237 unsterile cans, but only 13 with active spoilage. They stated that the presence of living bacteria has little significance as to the quality of the product when the can is opened, since some of the sterile cans were found on chemical examination to contain putrid and decomposed fish, while many of the cans from which bacteria were grown contained apparently sound fish.

Weinzirl (1919) examined bacteriologically 1018 samples of canned goods. He noted the condition of the container, appearance, consistency, odor and taste of the contents and the microscopic findings, as well as making gelatin and agar plate cultures and enrichment cultures in broth at 37 to 55° C. He listed the spoiled and suspected canned goods separately from those called commercial canned foods (evidently not spoiled), but stated that among the suspected cans were included some samples the contents of which were entirely sound. He found, as have other investigators, that sound canned foods are not always sterile, but may contain viable spores of bacteria.

- 1921 Bigelow (1921) discussed methods for the detection of spoilage in canned foods. He divided them into organoleptic (appearance, color, odor, taste) bacteriological, and chemical, and the examination of the can. He stated that when organoleptic examination shows abnormal products, bacteriological examination will often disclose

the reasons. The hanging drop sometimes gives evidence of spoilage before sterilization. Certain products of bacterial spoilage such as high acidity may be detected by chemical methods. He stated that a qualified analyst is better able to judge of the soundness of a sample by organoleptic examination than by examination by means of bacteriological and chemical methods.

Tillmans, Strohecker and Schütze (1921) modified the Tillmans and Mildner's oxygen consumption method for detection of incipient decomposition in meat. They devised two other tests. By their nitrate reduction test, meat in incipient decay gave no nitrate reaction after four hours' incubation. The decolorization of methylene blue in less than one hour was taken to indicate incipient decomposition.

- 1922 Clough (1922) found that, in general, when more than 1.5 mg. of indol per 100 gm. is found in canned salmon, a considerable degree of decomposition has taken place. He made quantitative determinations of indol in 544 cans of salmon. As some of these had a strong tainted odor and yet contained very little indol, the absence of indol cannot be taken as evidence that decomposition has not taken place. Rather close correlation was found between the number of bacteria present from day to day during spoilage and the indol content. The indol producing power of 299 different bacterial cultures from salmon was tested and gave only 31% positive tests. The volatile nitrogen increased from day to day during salmon decomposition, but owing to its probable formation during the cooking process it was not a suitable measure of decomposition in canned salmon. It was of value in raw salmon. The free fatty acids of salmon oils gave very unsatisfactory results as a measure of decomposition. Peterson (1922) made bacteriological and chemical analyses of normal and discolored kraut and concluded that discolored kraut was characterized by high alcohol content, low lactic acid, low sugar, and the presence of large numbers of wild yeasts. Savage, Hunwiche and Calder (1922), in a study of the bacteriology of canned meat and fish, concluded that sterility is not in itself a reliable test of soundness and that samples cannot be justifiably condemned merely because they are not sterile.
- 1923 Clark, Clough, Fellers and Shostrom (1923) described their systematic method of examining canned salmon. The work included physical, bacteriological and chemical examinations. Hunter and Linden (1923), in an investigation of oyster spoilage, found that the spoilage of shucked oysters is more or less definitely correlated with the hydrogen ion concentration of the oyster liquor. Oysters passing from good to stale changed from pH 6.1 to 5.6 while those with a pH of less than 5.0 were usually in an advanced stage of decomposition. Yeast counts, the number of lactic fermenting organisms and bacterial counts from the oyster meats were found too variable to be of significance in determining spoilage of oysters. Schoenholz, Esty and Meyer (1923), in studying correlation of toxin production and signs of spoilage in commercially canned vegetables and fruits inoculated with detoxified spores of *Bacillus botulinus*, felt that the association of physical signs of spoilage with toxicity

had been over-emphasized. Asparagus, beets, spinach and string beans were sometimes toxic without showing physical evidence of spoilage.

Thom (1923) stated that "a food product should be considered spoiled when a discriminating consumer, knowing its history or handling the 'raw' product, would refuse to eat it.

Wyant and Tweed (1923), in a description of 3 cans of so-called "flat sour" canned peas and inoculations into sterile cans of vegetables of four organisms isolated, used the following tests in examination for spoilage: gas production, color, appearance, odor, consistency, pH, and growth in shake cultures in dextrose agar at 37° C. and 65° C. They considered "flat sours" to be apparently normal cans whose contents had a pH below 5.8 without the production of gas or of only a very small quantity of gas.

- 1924 Bidault (1924) made twenty-four different chemical tests on canned meats which had stood from one to thirty-two years. He found a slight increase with age in the amount of ammoniacal nitrogen, amino nitrogen, especially that from tryptophan, and a large increase in volatile acids. The composition of the gases in the cans had changed, most of those packed ten years or more showing considerable hydrogen.

Broadhurst and Van Arsdale (1924) found that there was no close correlation between the rate of bacterial multiplication and hydrogen ion or acidity readings in food spoilage in the ice box. This was explained on the basis of mixed organisms growing in the food. They stated that "measurable chemical changes and by-products apparently lag far behind bacterial multiplication ratings or else mask each other." Unless organisms produce some marked objectionable change (musty odor, sourness), the bacteria may become innumerable before a given foodstuff will be rejected as food.

Fellers, Shostrom and Clark (1924) determined hydrogen sulfide production in bacterial cultures and canned gooseberries, salmon and shrimp. Twelve out of fifty-three organisms tested gave positive results. Canned gooseberries which had been sprayed while immature with lime-sulfur contained large quantities of H_2S when spoiled, but when normal, none. Under the conditions of the experiment no H_2S was liberated from decomposed salmon or shrimp. Murray (1924) tested methods of detecting incipient decomposition in foods. She found the reduction of methylene blue of greater value as a standard to indicate the time of initial decomposition than other methods tried. A positive nitrate reduction was obtained earlier than a great increase in bacterial count, but the test was somewhat too sensitive to indicate incipient food spoilage. No good results were obtained from change in titrable acidity or hydrogen ion concentration.

Thom and Hunter (1924) stated that chemical methods in detecting decomposition are occasionally effective but often exceedingly difficult to interpret. "The end products of decomposition resulting from many rotting processes occur in such small amounts and are so difficult to identify that decomposition must be excessive before it can be detected readily."

Tillmans and Otto (1924) found that the flesh of fish decomposes differently from that of mammals. Incipient decomposition in fish could be detected by the determination of NH_3 and amino N, though this method fails to indicate incipient decomposition of meat. Fish with more than .03% NH_3 or more than .1% amino N was starting to spoil. Oxygen absorption could be used to detect spoilage in either meat or fish. They found the reduction of potassium nitrate gave varying values, though reduction in less than four hours might always be considered a sign of incipient decomposition. The reduction of methylene blue was considered an indication of somewhat more advanced decomposition. If the methylene blue was reduced within an hour, decomposition was definitely indicated. The determination of peptone, carbonic acid, indol, soluble nitrogen, and the ability to combine with iodine furnished varying or negative results.

1925 Almy (1925) described a method for estimation of hydrogen sulfide in proteinaceous food products and found that H_2S was formed progressively during putrefaction of beef, pork and fish.

Arbenz (1925) used the consumption of oxygen and the reduction of methylene blue for detecting the degree of putrefaction of meat. Satisfactory results were obtained in testing beef, pork, veal, mutton, horse, mince meat, sausage, pigeon and several kinds of fish.

Association of Official Agricultural Chemists (1925) described official and tentative methods for the analysis of canned vegetables and for tomato products.

Clough, Shostrom and Clark (1925) tested thirty-five food products from sixty-six sources for indol and skatol. They found no skatol, but concluded that the presence of indol may be safely taken as an evidence of some degree of decomposition.

Clough, Shostrom and Clark (1925) gave the results of an experimental pack of salmon showing the correlation between the gases present and the condition of the fish. They found that hydrogen, when present, is a positive indication of decomposition, but its absence does not signify that no decomposition has taken place.

Dill (1925) investigated the post-mortem disappearance of glycogen as a possible index to spoilage in clams. Owing to the seasonable variation of glycogen content of the fresh clams the amount present could not be used as a criterion of the freshness of the clams when canned.

Esty and Stevenson (1925) published a detailed paper on the method and diagnosis in the examination of spoiled canned foods. They developed a routine method for bacterial examination of canned foods based on years of field and laboratory experience. They suggested correlations involving historical, bacteriologic and physical data. Their routine examination includes incubation of the samples to be tested, noting the condition of the container, cleaning and opening the container, inoculating into standard plain and dextrose broths pH 7 with brom cresol purple indicator and dextrose peptic digest beef heart medium covered with petrolatum and incubating the tubes for at least one week at 37 to 55° C., noting changes in hydrogen ion concentration of the canned product, microscopic examina-

tion of stained smears from the canned product, physical examination of the contents and examination of the cultures secured. With acid foods, as tomatoes and fruits, special media having tomato juice as a base and the incubation temperature of 35 to 37° C. are employed. Their paper discusses the significance of various findings in their relation to the cause of spoilage.

Sullivan (1925) reported canned beans from a lot containing flat sours being sent to four collaborators for examination. They were judged by vacuum, odor, taste, pH, cloudiness of liquid, counts of living bacteria and microscopic stains. One collaborator found that the acidity increased, the pH decreased, and the number of living organisms increased in the spoiled cans.

- 1926 Cameron and Esty (1926) applied the term "flat sour" only to those spoiled products which have a distinctly sour taste (pH value not markedly above 5.0) without the evidence of gas production. They found that when canned foods spoil as a result of understerilization, "swells" result from anaerobic activity and "flat sours" denote the presence of facultative anaerobic types. The 214 cultures of "flat sour" organisms studied belonged to the facultative thermophilic or the obligative thermophilic groups.

- 1927 Savage (1927) emphasized the importance of carrying out an examination in a systematic manner as a regular routine for the detection of spoilage in canned foods. He included in his routine analysis the examination of the unopened tin; sterilization of the tin before opening; culturing; noting the condition of the contents, including gas escape, appearance and odor; direct microscopic examination; and chemical tests as titrable acidity and examination for tin if desirable. In rare cases chemical tests for evidence of decomposition are indicated.

Wadsworth (1927) described the method used by the N. Y. State Department of Health for examination of food in cases suspected of poisoning with *Cl. botulinum* and *Bact. enteritidis*.

- 1928 Thompson (1928) described a quick method for detecting spoilage in packs of canned corn. The time of sampling, number of samples, and incubation temperatures and periods were specified. Flat sours were detected by adding a few drops of Brom cresol purple indicator to the corn, when a yellow color indicated sourness. The thermophilic anaerobes were indicated by swelling of the cans on incubation at 130° F. Sulphide spoilage was shown by darkly discolored kernels and the odor of H_2S .

CHEMICAL TESTING OF NICOTINE DUSTS*

W. R. HARLAN AND R. M. HIXON

From the Laboratory of Plant Chemistry, Iowa State College.

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In a preceding publication (1927) an "unaccountable loss of nicotine" was reported for a series of dusts. A number of similar observations have been reported by other workers. McDonnell and Young (1925) have pointed out that there is considerable loss of nicotine from various dusts even when stored in sealed containers for various periods of time. Thatcher and Streeter (1923) have also reported significant losses in sealed containers. In view of these conclusions in the literature, decomposition was presumed to be the explanation of the previous results.

The following paper presents evidence to show that bentonite adsorbs nicotine so firmly that it cannot be quantitatively extracted from the dust with ether or water. It can, however, be quantitatively recovered by direct steam distillation of the dust. It is apparent that when any dust shows this abnormally strong adsorption that the interpretation of the "unaccountable loss of nicotine" in other dusts as due to decomposition becomes doubtful without further evidence of the precision of the particular analytical method used.

Decomposition of nicotine in the vapor phase has also been reported by de Ong (1923). In the previous publication it was found impossible to establish equilibrium conditions in the vapor over the dust. In the following data this is shown to be due to adsorption of nicotine vapor by rubber. An apparatus constructed entirely of glass using concentrations of nicotine vapor in equilibrium with liquid nicotine and nicotine dusts showed no decrease in concentration of the nicotine in the air mixture that could be detected over periods of forty minutes exposure. The inability to maintain such vapor concentrations has been the basis of evidence for decomposition of nicotine in the vapor phase.

It is concluded that any experimental method which permits the contact of nicotine vapor with rubber (i. e. storage of nicotine dusts in rubber stoppered bottles) or which reports the analysis of a dust by extraction with either ether or water without quantitative recovery cannot be interpreted as due to decomposition of nicotine without further evidence.

ADSORPTION OF NICOTINE BY BENTONITE

Previous to preparing a dust, the bentonite was extracted with ether to remove any ether-soluble products. The nicotine used was a colorless oil having a boiling point of 247° C. at 730 mm. and a density of 1.010 at 20° C. A dust containing 3% nicotine was prepared and samples allowed to remain exposed to the air for several days (nicotine is comparatively

*These studies were made possible through a Fellowship maintained by the Tobacco By-Products and Chemical Corporation.

non-volatile from bentonite dusts, the odor of nicotine completely disappearing in three days in closed containers) while other samples were kept in tightly stoppered bottles.

The samples of nicotine-bentonite dust were then extracted with ether for twenty-four hours in a Soxhlet extractor. In both the dusts exposed to the air and those in stoppered containers only 10% to 20% of the nicotine originally present was obtained by ether extraction. An examination of this ether extract failed to reveal any decomposition products of nicotine. The ether-soluble oil previously reported was found to have come from outside contamination during the extraction.

The ether-extracted dust was steam distilled and in all cases the remainder of the nicotine was quantitatively recovered. An examination of the steam distillate showed no decomposition products.

The amount of nicotine absorbed or held by bentonite is approximately a constant amount regardless of the concentration of nicotine in the dust. In a 3% dust 12% of the nicotine is extracted by ether, in a 6% dust 54% is extracted and in a 14% dust 80% is extracted; that is, in a 3% dust 0.0264 gm. of nicotine is retained per gram of bentonite, in a 6% dust 0.0276 gm. is retained and in a 14% dust 0.0280 gm. is retained. These data were obtained by extracting the nicotine-bentonite dusts for twelve hours in a Soxhlet extractor. The addition of hydrated lime (10% to 20%) increases the amount of nicotine extracted by ether.

Very little nicotine is obtained in the filtrate when a 3% nicotine-bentonite dust is washed with water. It is also noteworthy that considerable heat is evolved when pure nicotine is added to bentonite, which is indicative of an adsorption or union between the bentonite and nicotine.

The fact that there is no perceptible odor of nicotine in a 3% nicotine-bentonite dust two or three days after preparation when kept in tightly stoppered bottles, the fact of the insolubility of nicotine in ether and water, and the fact of the relatively large heat of adsorption when pure nicotine is added to bentonite indicate that the degree of adsorption is so great that it approaches a loose chemical union.

Bentonite and hydrated lime dusts have shown no significant loss of nicotine (less than 0.1%) when kept in tightly stoppered bottles for the past six months. It should be pointed out, however, that the silicotungstic acid method of analysis is a poor criterion for decomposition of nicotine. Any compound which is volatile with steam and similar in structure to nicotine will be precipitated along with nicotine by silicotungstic acid. Thus only radical changes in the nicotine molecule (which are improbable in dusts) or the formation of compounds non-volatile with steam will be noted by the present analytical methods.

ADSORPTION OF NICOTINE IN VAPOR STATE

It is stated by de Ong (1923) that if nicotine vapor diffuses in the air it is soon oxidized. As evidence of this he cites an experiment in which air was aspirated through a nicotine solution and the nicotine vapor passed through two or three feet of rubber tubing. Only a trace of nicotine vapor was recovered in the washing solution at the end of the tube, while a close correlation was found between the amount of nicotine recovered at the top of the tube and the concentration of the original solution.

Chemically, nicotine is comparatively stable toward weak oxidizing agents. It is a common characteristic of many nitrogenous bases with a pyridine nucleus to darken when exposed to light or air without any appreciable decomposition taking place. Nicotine is oxidized to oxynicotine by a 2½% solution of H_2O_2 . This is the most logical oxidation product of nicotine with air acting as the oxidizing medium. No trace of this compound has been found in dusts exposed to the air or in nicotine vapor-air mixtures.

Nicotine vapor from a nicotine-hydrated lime dust (using apparatus described by Hixon and Drake) was passed through a series of 3-2 liter glass bottles. These bottles were closed with rubber stoppers with an inlet and outlet glass tube, these tubes being connected together with rubber tubing. Air was passed over the dusts at six liters per hour. One hour would then elapse from the time the nicotine entered the series of bottles until it was absorbed for analysis by washing the gases in 2N H_2SO_4 with a series of bubblers. Time was allowed in all cases for any ordinary absorption on the walls of the bottles to take place before any analyses were made. Analyses of the nicotine concentration were made before and after the vapor had passed through this series of bottles. A large decrease in concentration was always noted. This difference in nicotine concentration was also noted biologically. Rice weevil (*Calendra oryzae* L.) were placed in each of the bottles in the series. Much larger kills were always obtained in the bottles nearest the source of nicotine vapor.

These observations are in harmony with de Ong's conclusions mentioned above, but when an entire glass train with no rubber stoppers or connections was inserted no such discrepancies were found. In table I data are given upon which these statements are based. In numbers 1 and 2 the nicotine vapor was passed through a train containing rubber connections, while in numbers 3, 4 and 5 the vapor was passed through an entire glass train. One hour was required for the nicotine vapor to pass through the train in experiments number 1 and 2, while only forty minutes was required in numbers 3, 4 and 5.

TABLE I.

Experiment Number	Conc. mgs. nicotine per 10 liters air at beginning of train.	Conc. mgs. nicotine per 10 liters air at end of train.	% nicotine not accounted for
1	2.56	1.52	—40.6%
2	1.56	0.90	—42.3%
3	2.67	2.66	— 0.4%
4	2.99	2.97	— 0.6%
5	2.76	2.78	+ 0.7%

These experiments were duplicated using pure nicotine as the source of nicotine vapor in place of the dusts, with similar results. These data will be reported in a subsequent publication on the volatility of nicotine. These data show that there is no rapid oxidation of nicotine vapor as suggested by de Ong, his conclusions being due to the adsorption of nicotine by rubber.

SUMMARY

1. A careful chemical study has failed to reveal any decomposition of nicotine either in dusts or in the vapor phase.

2. Dusts such as bentonite adsorb nicotine so strongly that it cannot be extracted by either water or ether.

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THE PREPARATION OF ETHYL β -FURYLACRYLATE FROM FURFURAL¹

BY HENRY GILMAN, R. E. BROWN AND H. L. JONES

From the Chemical Laboratory of Iowa State College

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INTRODUCTION

There is a large commercial demand for β -furylacrylic acid or its sodium salt. The satisfaction of this demand is contingent on the preparation of the acid or its salt at a so-called reasonable price. All commercially feasible methods for the preparation of this compound must, of necessity, start with furfural. Accordingly, a study has been made of the preparation of the acid from furfural. The stipulated prerequisites have been met as a result of the investigation reported here of the optimal conditions for the preparation of ethyl β -furylacrylate. The ester is hydrolyzed with great smoothness to give the desired acid or its salt.

The ester was first prepared by the esterification of furylacrylic acid by means of ethyl alcohol and concentrated sulfuric acid². Then Claisen³ prepared it in a 36.1% yield, by the use of the general reaction which now bears his name, from furfural, ethyl acetate and sodium. As a result of a series of experiments it is now possible to prepare the ester by the Claisen condensation in yields as high as 63.3%.

EXPERIMENTAL

The following details of a typical run incorporate the more practical conditions for the preparation of the ester. The results obtained in some other runs under varying conditions are incorporated in Table I.

In a two-liter, three-neck, round-bottom flask fitted with an efficient stirrer and a thermometer are placed 29 grams (1.25 atoms) of well-powdered sodium⁴. The flask is then cooled in a freezing mixture, and 455 c.c. or 410 g. (4.66 moles) of cold, absolute ethyl acetate is added⁵. As soon as the ester has been added, stirring is started and when the mixture reaches -10° then 96 g. (1 mole) of redistilled furfural is added drop by drop from a separatory funnel. The appearance of a reddish-brown color on the particles of sodium and a rise in temperature indicate that the reaction has started⁶. The temperature of the mixture should not be allowed

¹ This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge help from the Industrial Science Research Fund for the defrayal of expenses incurred in this investigation.

² Marckwald, Ber., 21, 1398 (1888).

³ Claisen, Ber., 24, 143 (1891). It has also been used in other studies by Asahina and Shibata, J. Pharm. Soc. Japan, No. 423, 391-9 (C. A. 11, 2457, 1917). The abstract of this article does not mention the method of preparation.

to go above -5° . After all the furfural has been added (about two hours) the stirring is continued for half an hour with the flask still surrounded with the freezing mixture.

The freezing mixture is removed and the stirring is continued for another hour at room temperature. Then, through the separatory funnel, there is added 120 c.c. or 125 g. (2.1 moles) of glacial acetic acid⁷, followed by about 500 c.c. of water.

The ethyl acetate layer is separated, and the aqueous solution is extracted with 50 c.c. of ethyl acetate. After washing the combined ester portions with 100 c.c. of water, it is dried with about 75 g. of anhydrous sodium sulfate. The ethyl acetate is then distilled from a water bath, and the ethyl furlacrylate at reduced pressure from an oil bath. The yield of ethyl furlacrylate distilling as a light yellow oil at $115-119^{\circ}/14$ mm. is 100 g. or 60% of the theoretical amount.

General Comments: The use of much lower temperatures than those just specified did not significantly improve the yield of ester, but added considerably to the difficulties of the preparation by greatly extending the time of addition of furfural and by making the process of cooling very laborious. A run between -14° and -15° gave a 63.3% yield of ester. (See Table I.) If the temperature is held between 0° and 5° the yields are consistently above 50%. Substitution of part of the ethyl acetate with invert solvents like petroleum ether gave very poor yields, and the yield was also decreased by the use of smaller quantities of ethyl acetate than those specified above.

In Claisen's experiments³ where he obtained a 36.1% yield the quantities of materials used were as follows: one mole of furfural, one atom of sodium and six moles of ethyl acetate.

The authors wish to thank George Wright for help in some of the experiments. They are also grateful to the Miner Laboratories of Chicago for liberal supplies of furfural.

⁴ The powdered sodium can be conveniently prepared by shaking molten sodium under hot xylene in a tightly stoppered 500 cc. round-bottom Pyrex flask. (See "Laboratory Manual of Organic Chemistry" by Fischer, p. 139, 2nd edition, published by John Wiley and Sons). The sodium can also be powdered by melting it under xylene in the flask in which the run is carried out. As soon as the sodium has melted, the stirrer is started and kept running until the xylene has cooled. An efficient stirrer and rapid stirring are necessary to produce finely divided sodium. The xylene may be carefully decanted after it is cold.

⁵ Absolute ethyl acetate is necessary for this preparation. The grade supplied by the U. S. Industrial Alcohol Company is quite satisfactory. The purification of ethyl acetate as described by Gattermann ("Practical Methods of Organic Chemistry" p. 179, third edition, published by MacMillan and Company) and others does not seem to give as good yields as can be obtained by the use of the above mentioned grade of ethyl acetate.

⁶ If a rise in temperature is not noted after about one cc. of furfural has been added, the stirring should be stopped until bubbles rise from the sodium. Stirring is then resumed. The addition of furfural should be slow.

⁷ When the glacial acetic acid is added, the mixture in the flask becomes almost solid, and care must be taken to stir it well so that all of the excess sodium is destroyed; otherwise a fire may result when water is added.

TABLE I.

Furfural		Sodium		Ethyl Acetate		Temp.	% Yield	Hours stirring at room temp.
g.	moles	g	atoms	cc.	moles			
96	1	29	1.25	455	4.66	0°- 5°	48.2	0
96	1	29	1.25	455	4.66	0°- 5°	48.2	0
96	1	29	1.25	455	4.66	0°- 5°	52.1	0
96	1	29	1.25	455	4.66	0°- 5°	56.4	2
96	1	29	1.25	455	4.66	-10°- 4°	60.3	1
96	1	29	1.25	455	4.66	-15°-14°	60.3	2
96	1	29	1.25	455	4.66	-15°-14°	63.3	2.5
192	2	58	2.5	455	4.66	-10°- 5°	51	2
96	1	29	1.25	147	1.5	-15°-10°	25	2
96	1	29	1.25	195	2	-10°- 5°	45	2

SUMMARY

Some optimal conditions have been described for the preparation of ethyl β -furylacrylate from furfural, sodium and ethyl acetate.

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